Synergy H1

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Agilent
Trusted Answers

BioTek®
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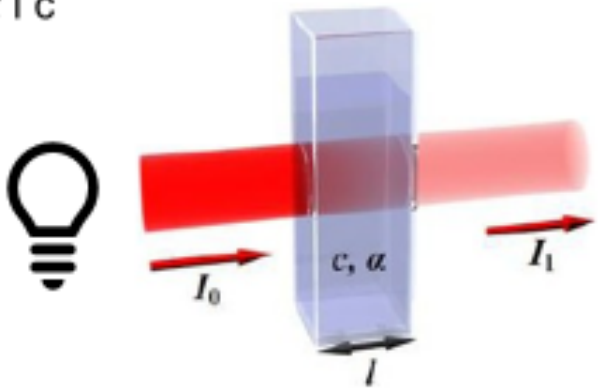
Microplate Reader and Absorbance

微量盤分析儀與吸光值

比爾定律 Beer's law

當光穿透樣品溶液時，光的吸收度(A)與吸收係數(ε)、光徑長(l)、濃度(c) 三者均呈正比：A=ε l c

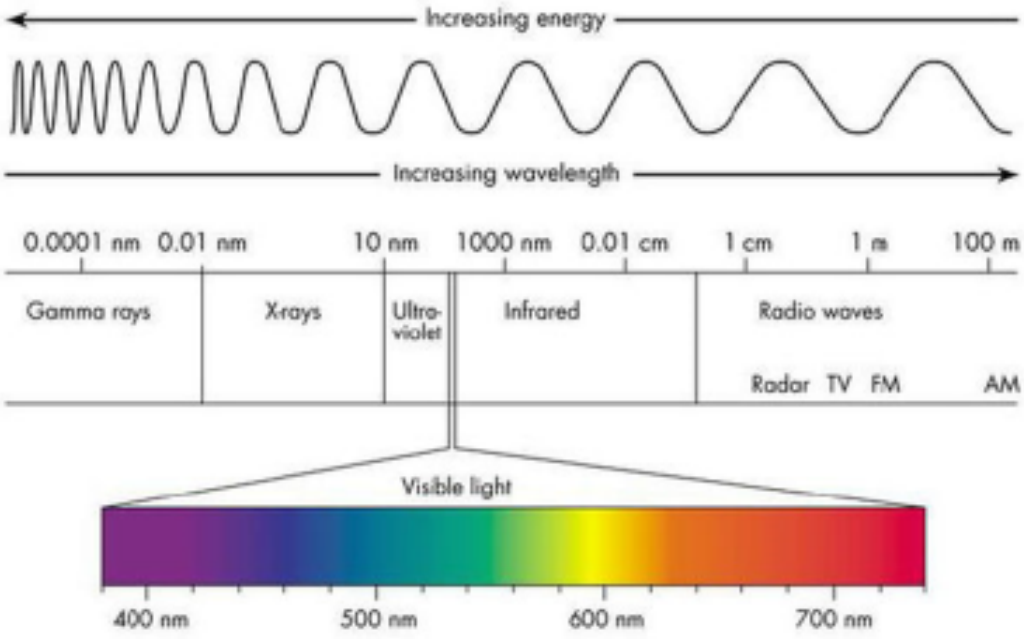


原本光入射線強度I_0，穿透光線強度變為I_l，此時光的穿透度為T (Transmittance)
定義光的被吸收度A

$$A = -\log T = -\log \frac{I_l}{I_0}$$

OD值 Optical Density

每種物質有其特定的吸收波長
一物質在某特定波長及固定距離下對於該光源的吸收值



濃度計算範例

$A=\varepsilon l c$

利用分光光度計在260 nm直接定量

若OD(吸光值) = 0.8 ，已知 ε(常數) = 0.02 mg/ml l(光徑) = 1 cm

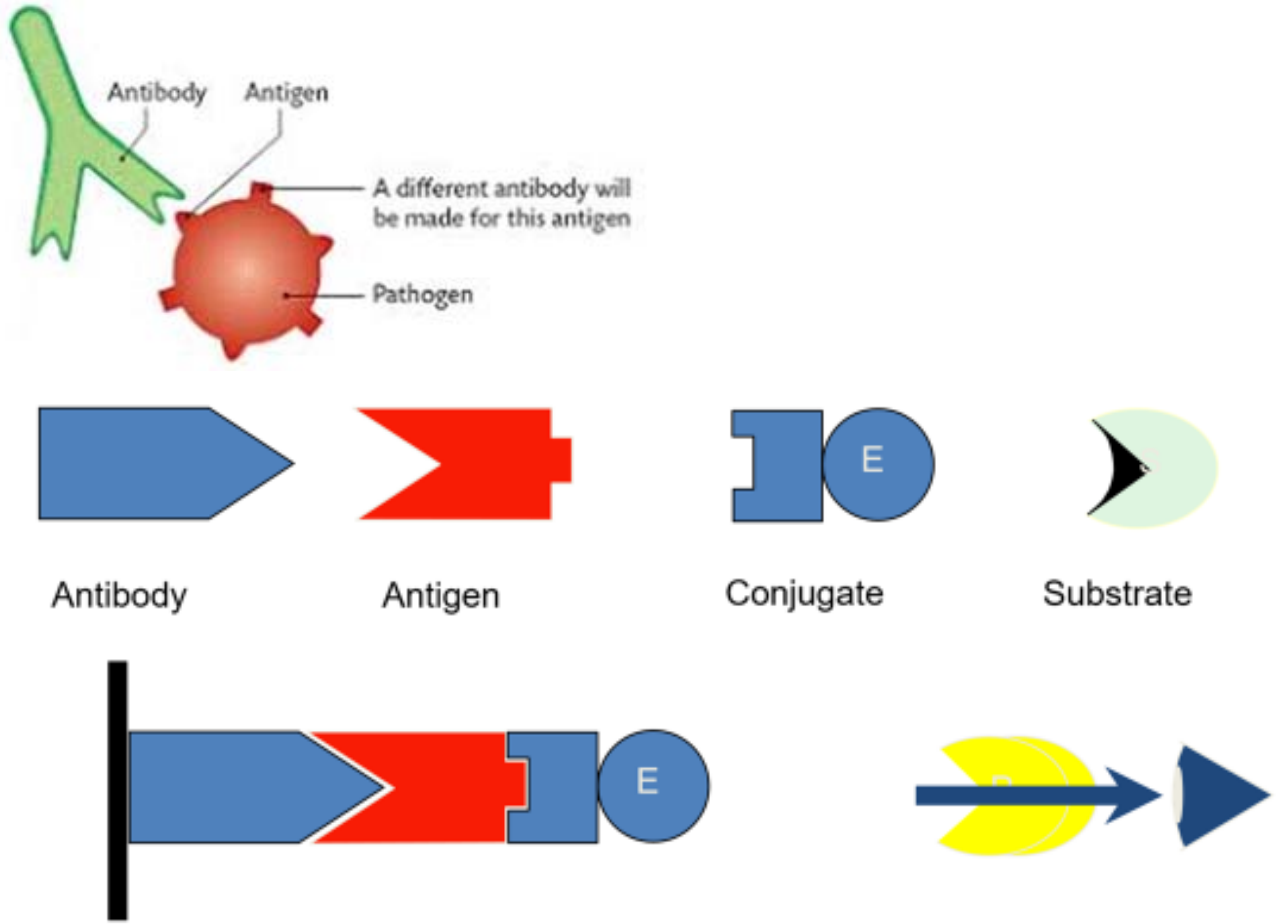
公式 $A= \varepsilon l c$

因此 0.8 = 0.02 x 1 x c

C = 0.8 x 50ug/ml = 40 ug/ml

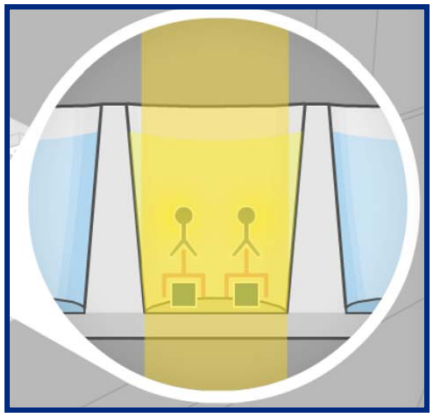
ELISA檢測原理

- 專一性抗原抗體反應，依據反應物顯色深淺進行定性或定量分析

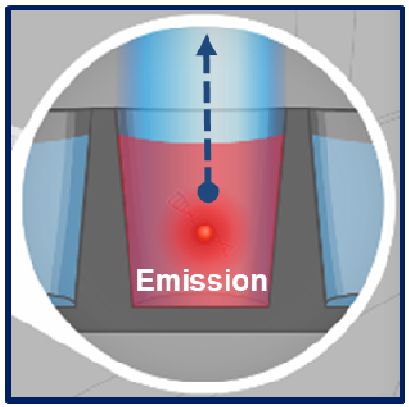


偵測方式

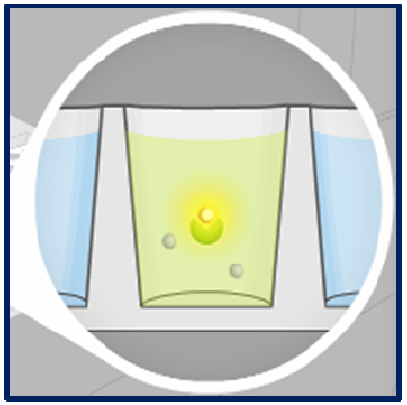
- 吸收光
- 螢光
- 冷光



Absorbance
吸收光

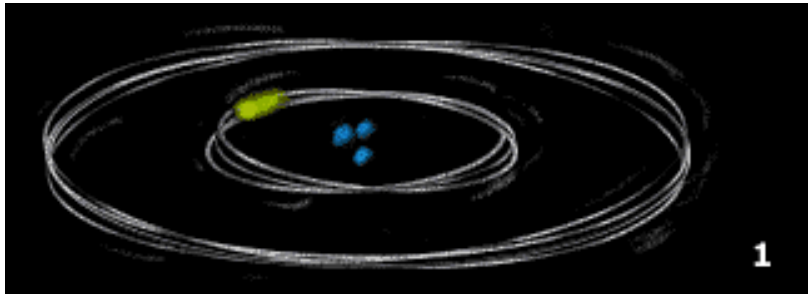


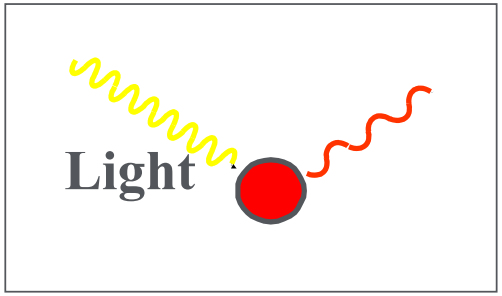
Fluorescence
螢光

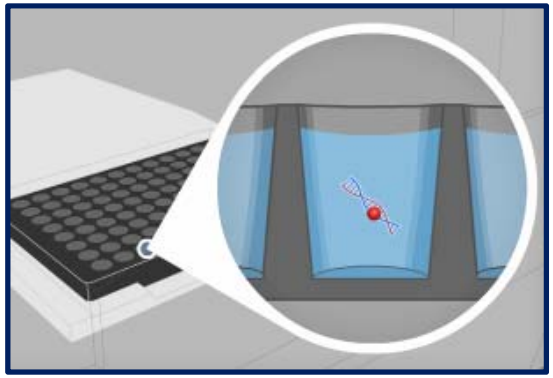


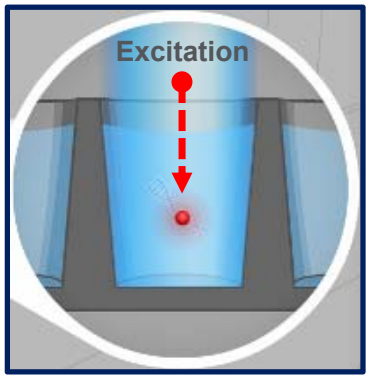
Luminescence
冷光

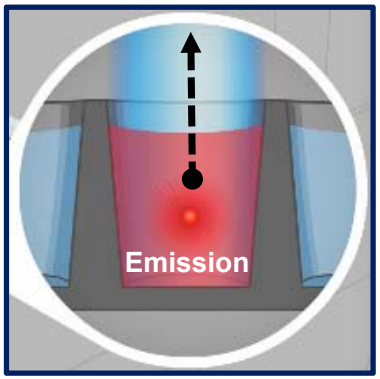
螢光原理與應用







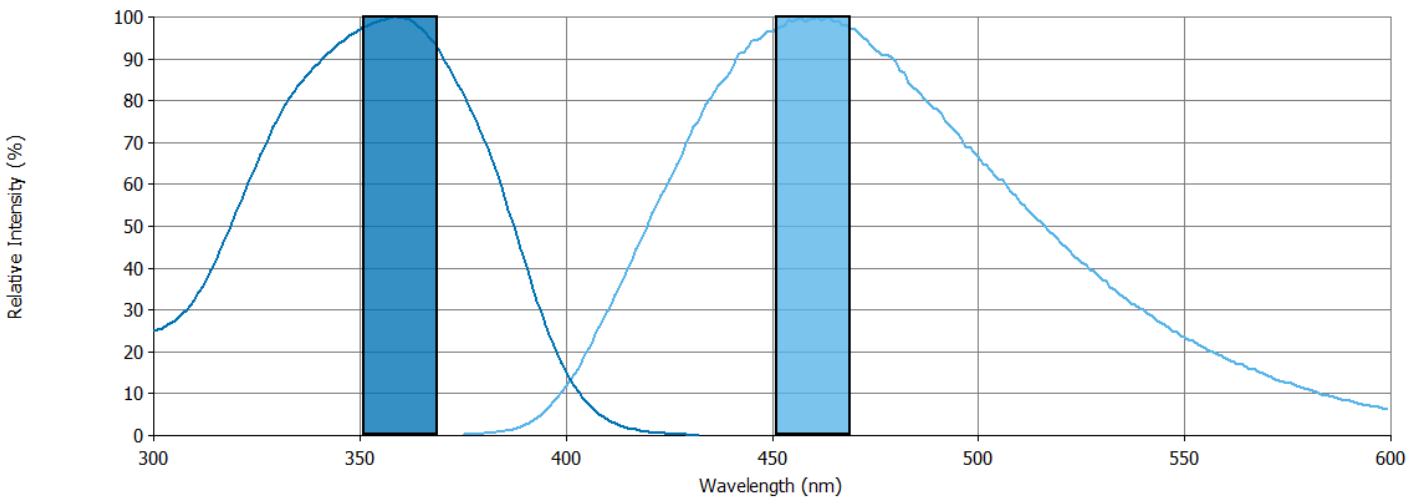




Unit：RFU (Relative Fluorescence Units)

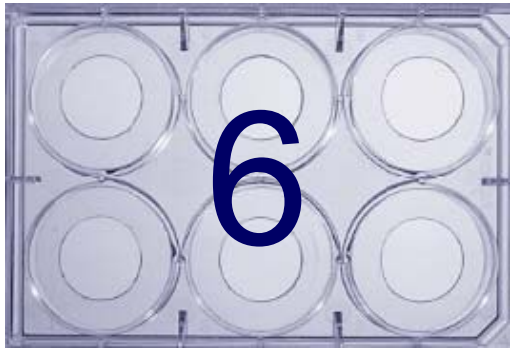
螢光原理與應用

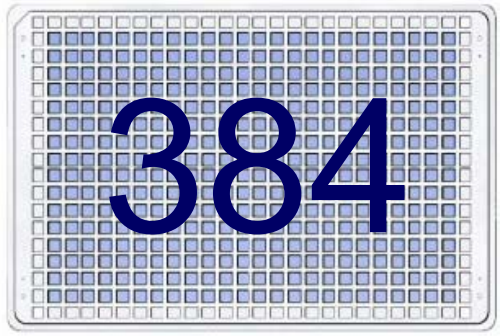
- Excitation 激發光
  - 光源激發螢光分子
- Emission 散射光
  - 螢光分子產生散射光
- 儀器偵測的是?
  - Emission激發光的數值
- Bandpass?
  - 波長可通過的範圍
- 460/40代表?
  - 440~480 nm範圍可通過濾鏡



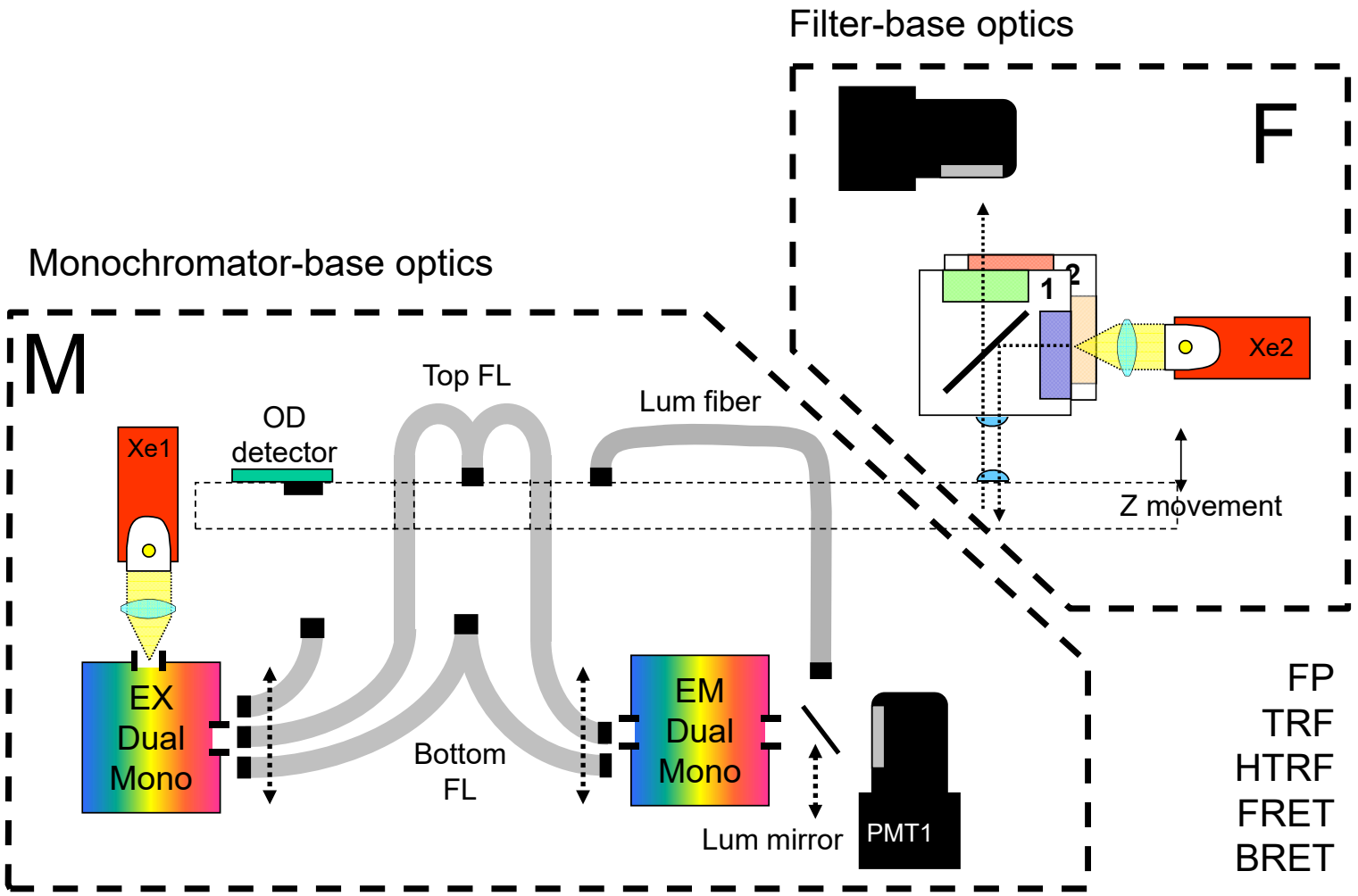
Synergy H1

微量盤類型

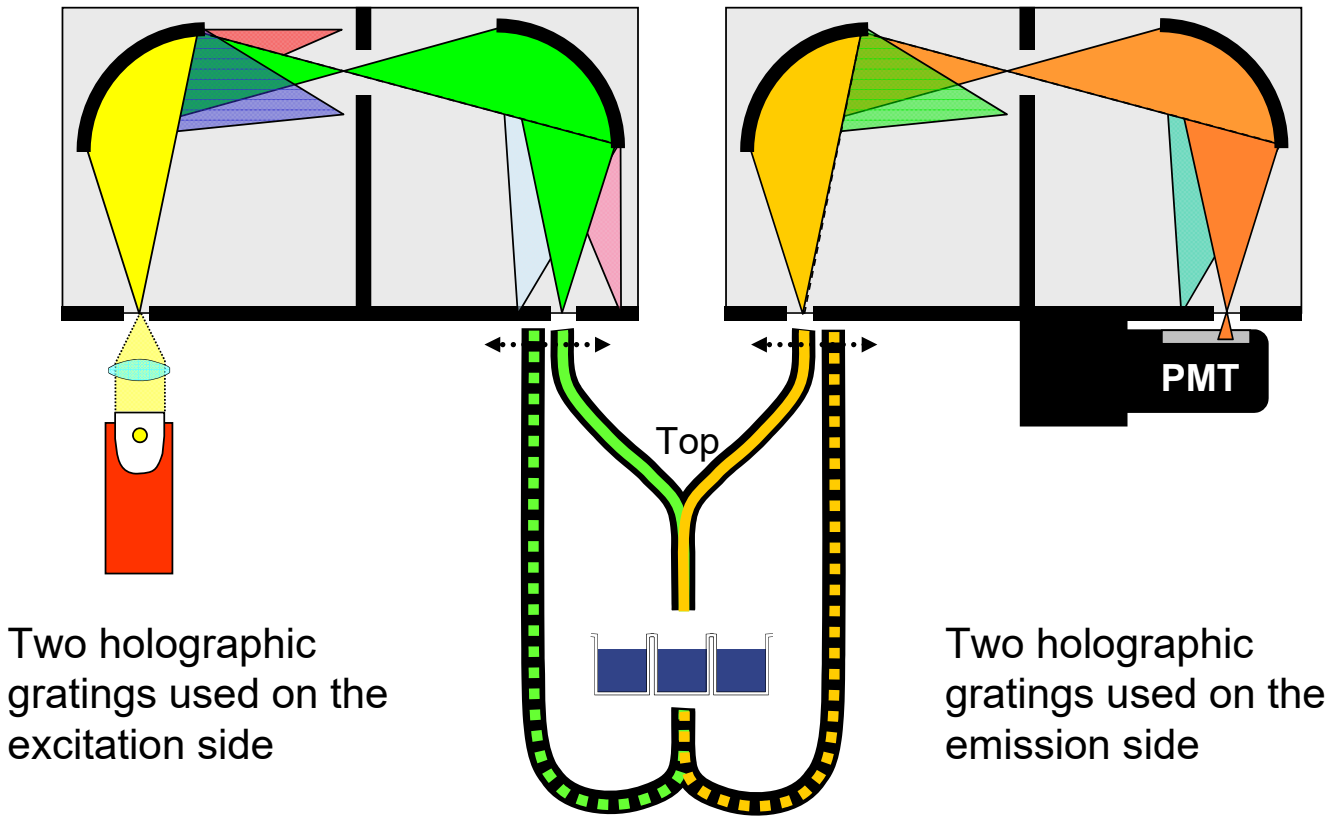




偵測模組


Filter-base optics
F
1
2
Xe2
Monochromator-base optics
M
Top FL
Lum fiber
OD
detector
Xe1
Z movement
EX
Dual
Mono
EM
Dual
Mono
Bottom
FL
Lum mirror
PMT1
FP
TRF
HTRF
FRET
BRET

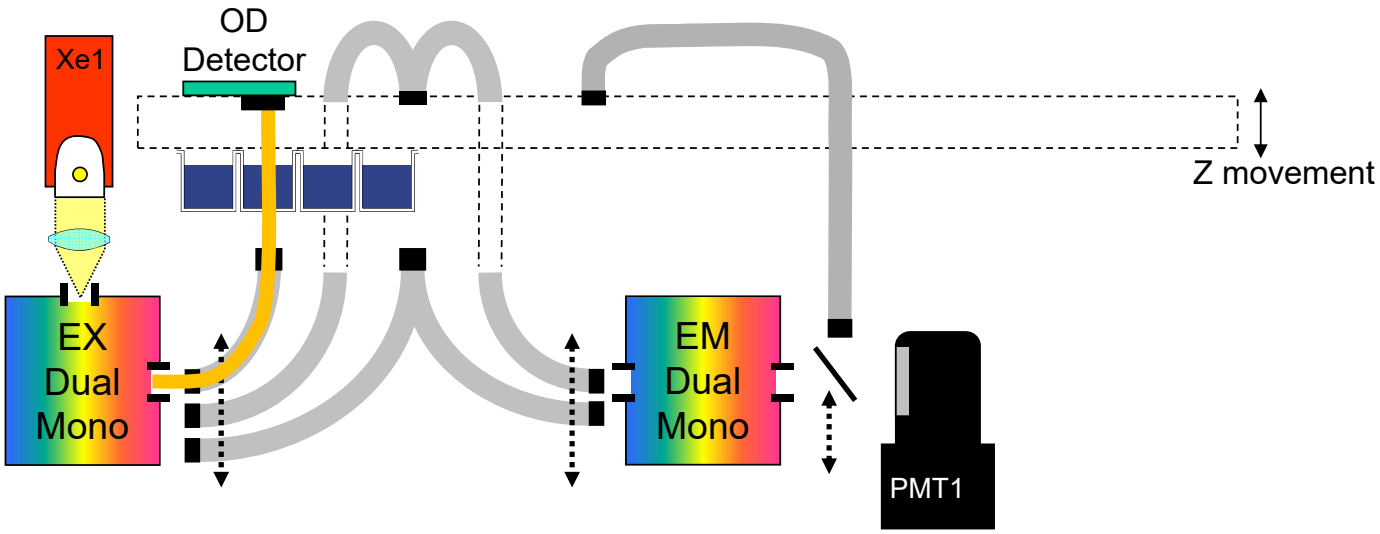
Quadruple Grating Optical Architecture



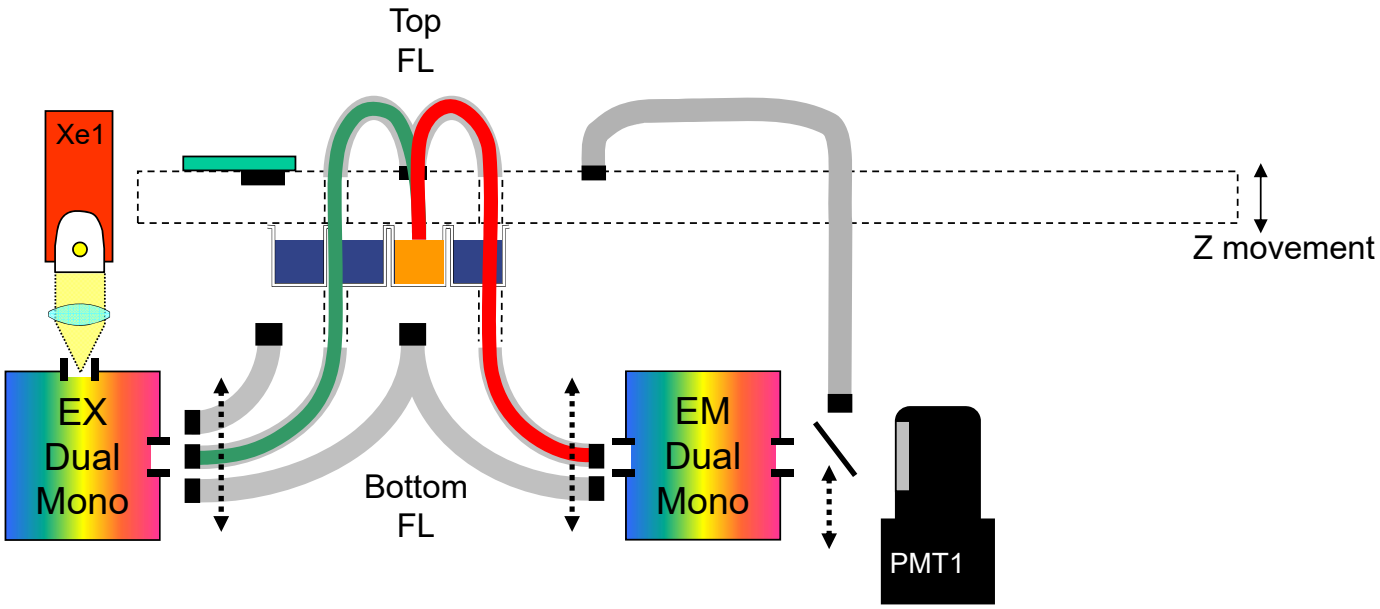
Two holographic gratings used on the excitation side

Two holographic gratings used on the emission side

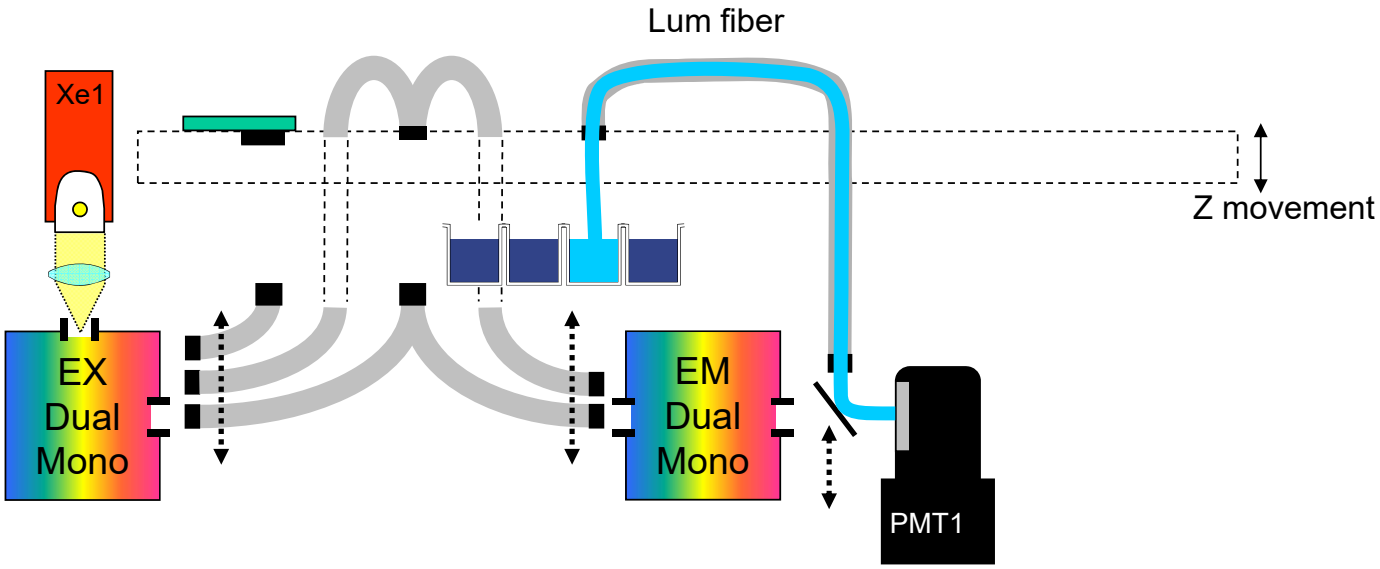
吸收光偵測路徑



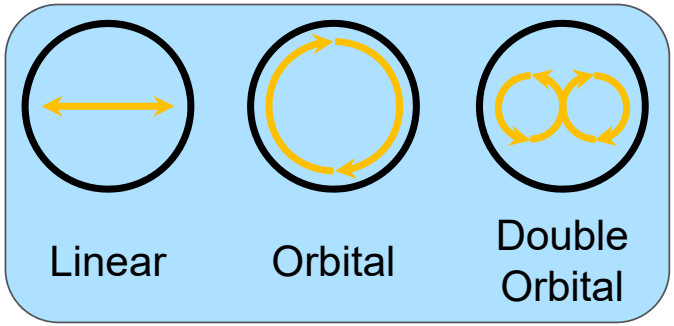
螢光偵測路徑

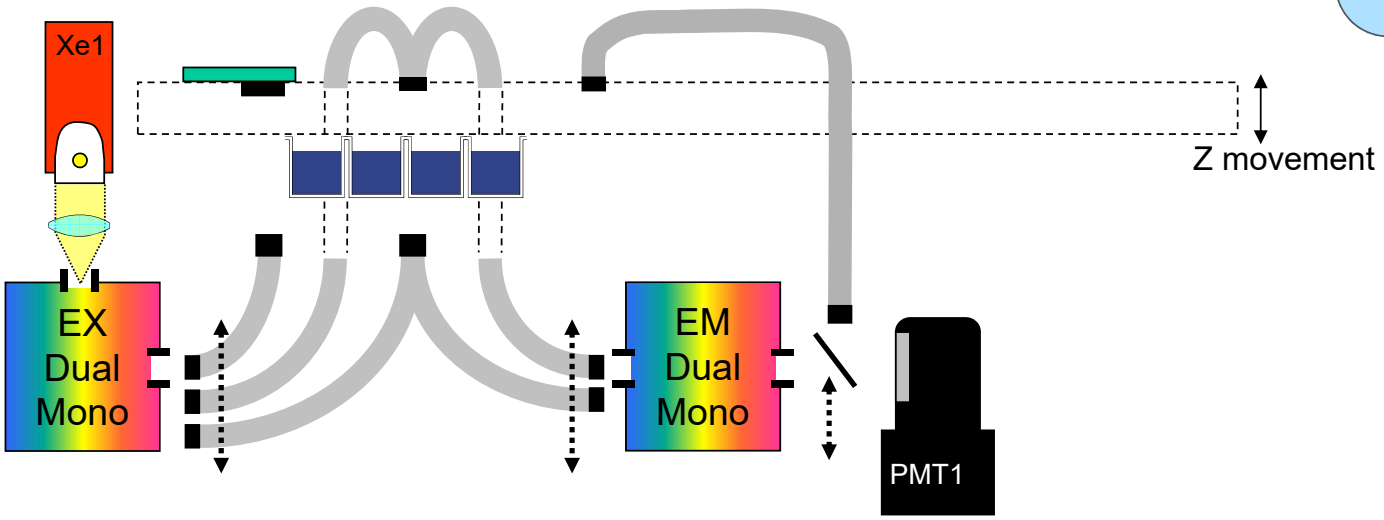


冷光偵測路徑

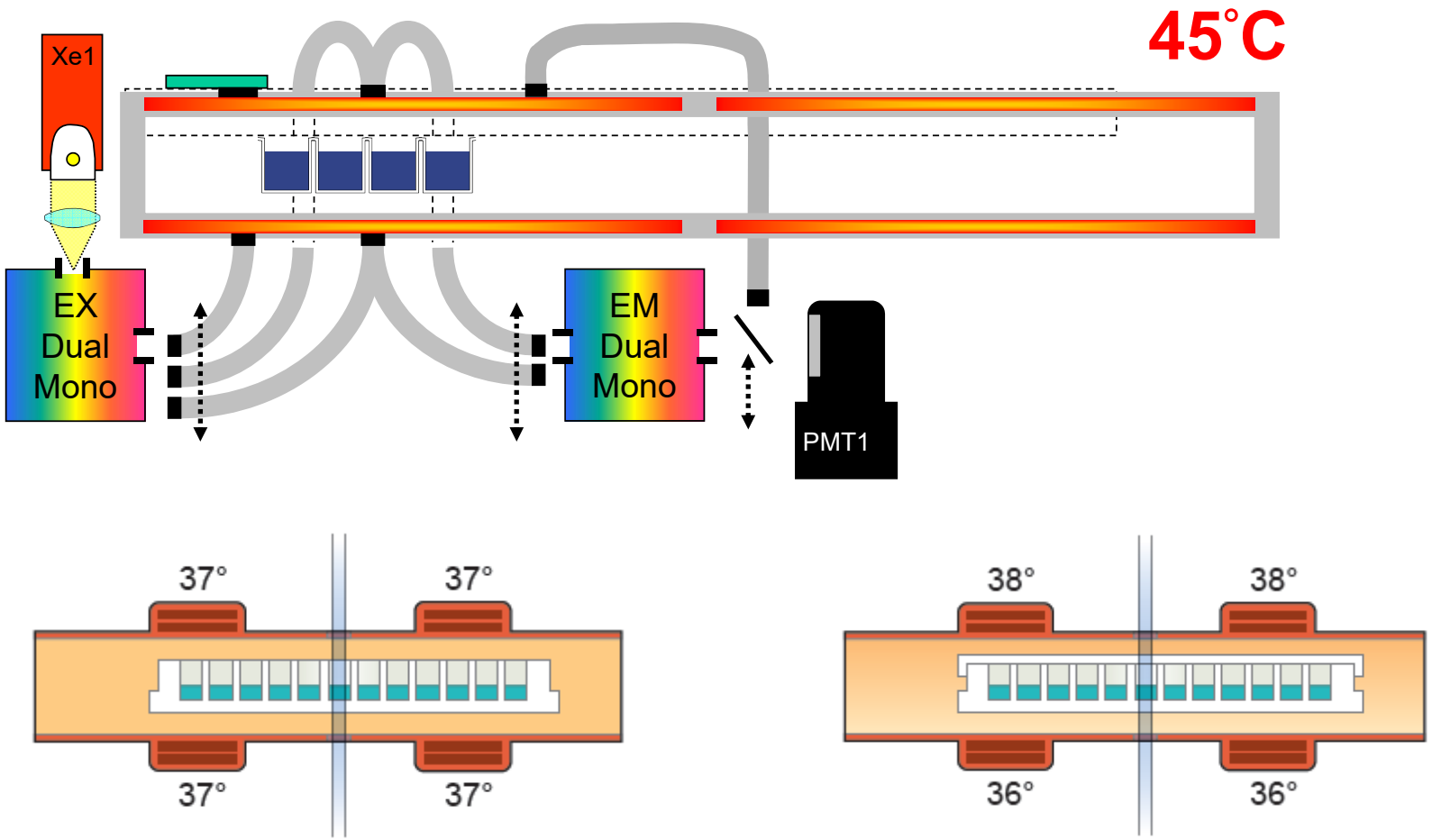


震盪功能


Linear
Orbital
Double
Orbital

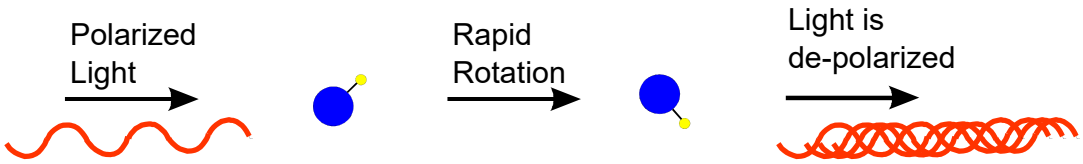

Xe1
EX
Dual
Mono
EM
Dual
Mono
PMT1
Z movement

溫度控制: 溫度梯度防止水氣凝結

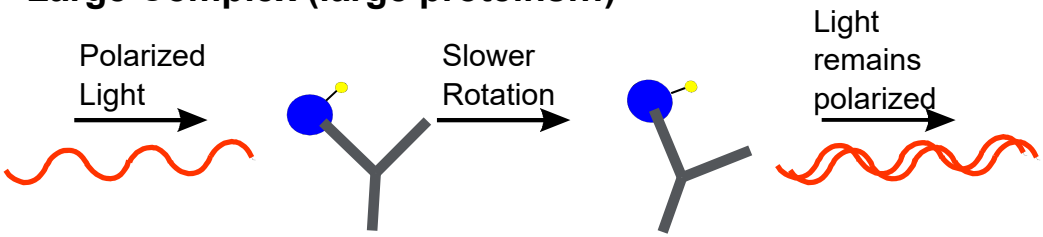


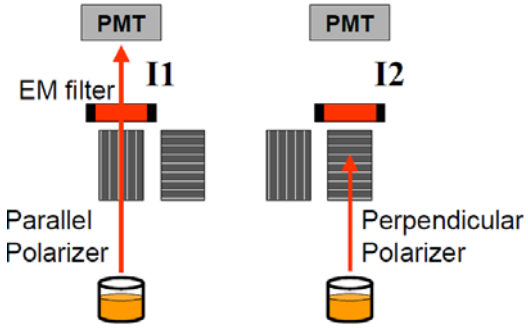
Fluorescence Polarization 偏極化螢光

- **Small Molecule (up to 1 kDa)**



- **Large Complex (large proteins…)**





$$P = \frac{I1 - I2}{I1 + I2}$$

Filter

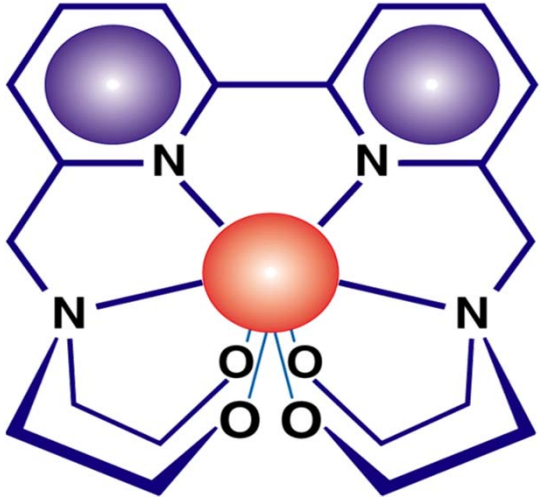
Polarizer

TRF 時差式螢光

Time-Resolved Fluorescence

Binding Arm

- Isothiocyanate (ITC)
- Iodoacetamido (IA)
- Amino
- Dichlorotriazine (DTA)



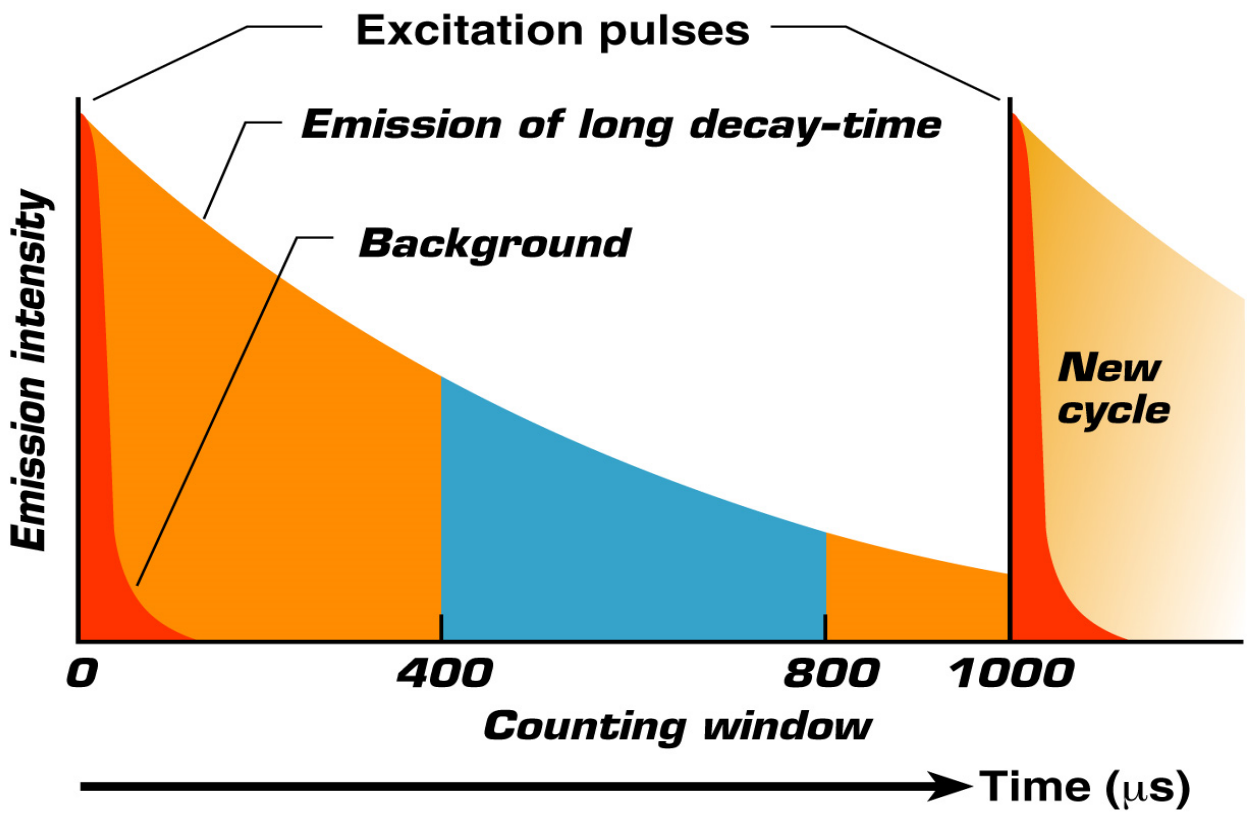
Chelate

- N1 (DTTA)
- DTPA
- W2014
- W1024
- W8044
- W14016

Sc														
Y														
La	Ce	Pr	Nd	Pm	**Sm**	**Eu**	Gd	**Tb**	**Dy**	Ho	Er	Tm	Yb	Lu
Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

TRF 時差式螢光

Time-Resolved Fluorescence



- ✓ Filter
- ✓ Monochromator

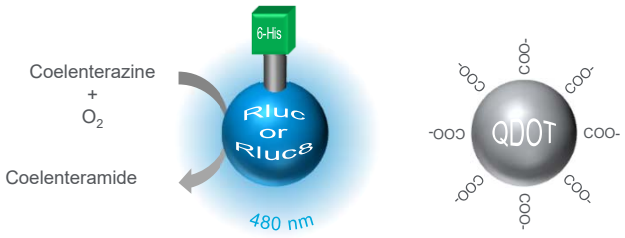
Background fluorescence is very low due to the time delay between excitation and plate reading

FRET 螢光共振能量傳遞

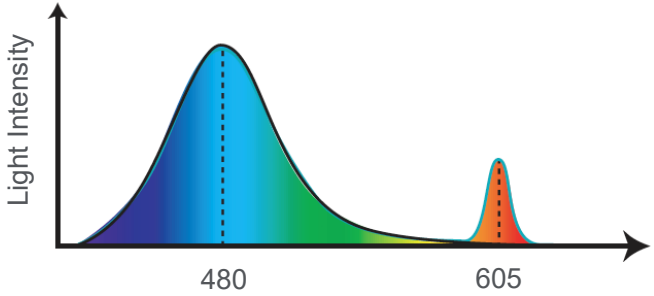
Fluorescence Resonance Energy Transfer

- ✓ Filter
- ✓ Monochromator

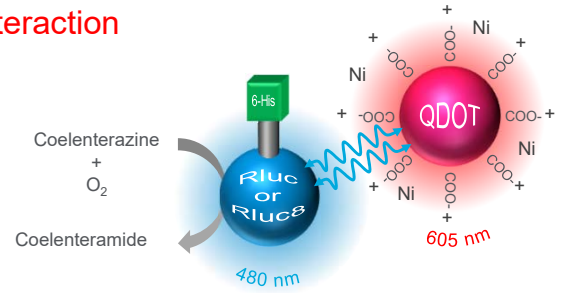
No Interaction



Spectral Properties



Interaction

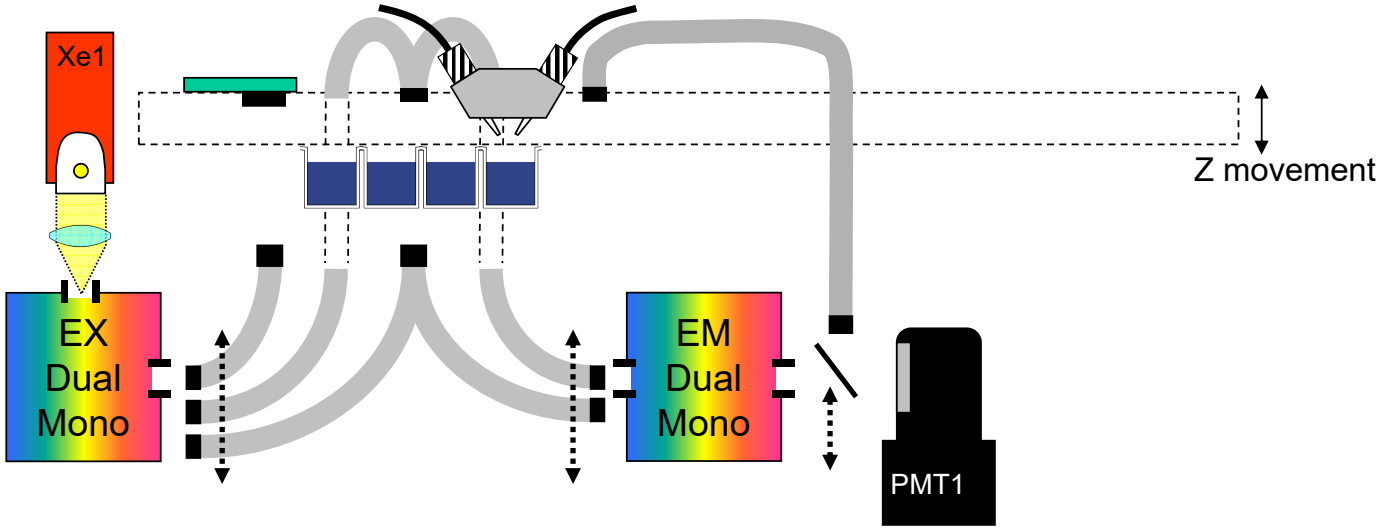


Other Compatible Acceptors
Other QDs

EXAMPLE APPLICATION

- Detection of proteases

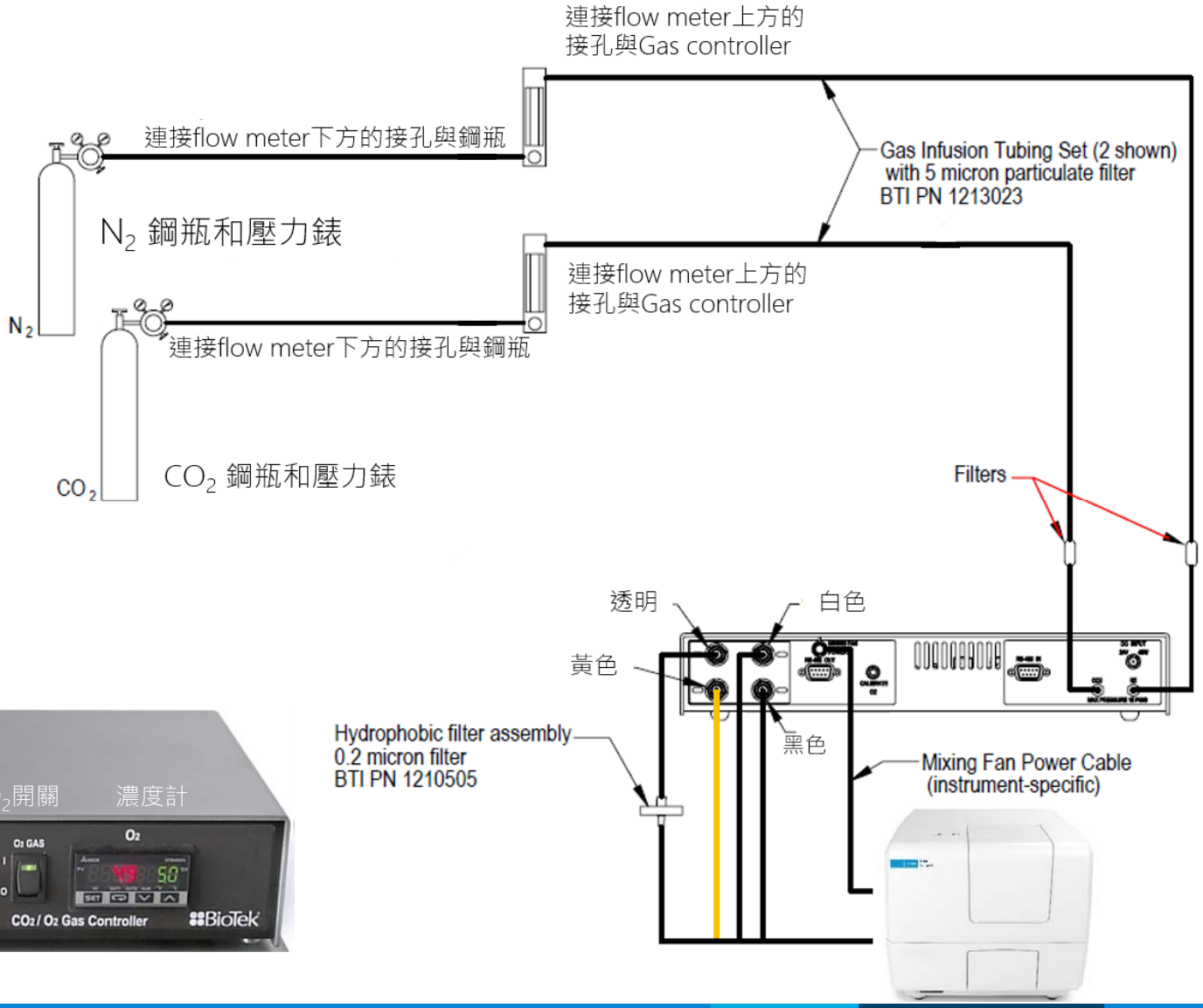
分注器



Number of Injectors:	2
Supported labware:	6- to 384-well microplates, Petri and cell culture dishes
Volume range:	5 – 1000 µL in 1 µL increments
Dead volume:	<1.1 ml with back flush (Synergy and Cytation) <1.65 mL with back flush (Lionheart FX)
Dispense accuracy:	+1 µL or 2%
Dispense precision:	<2% at 50 - 200 µL

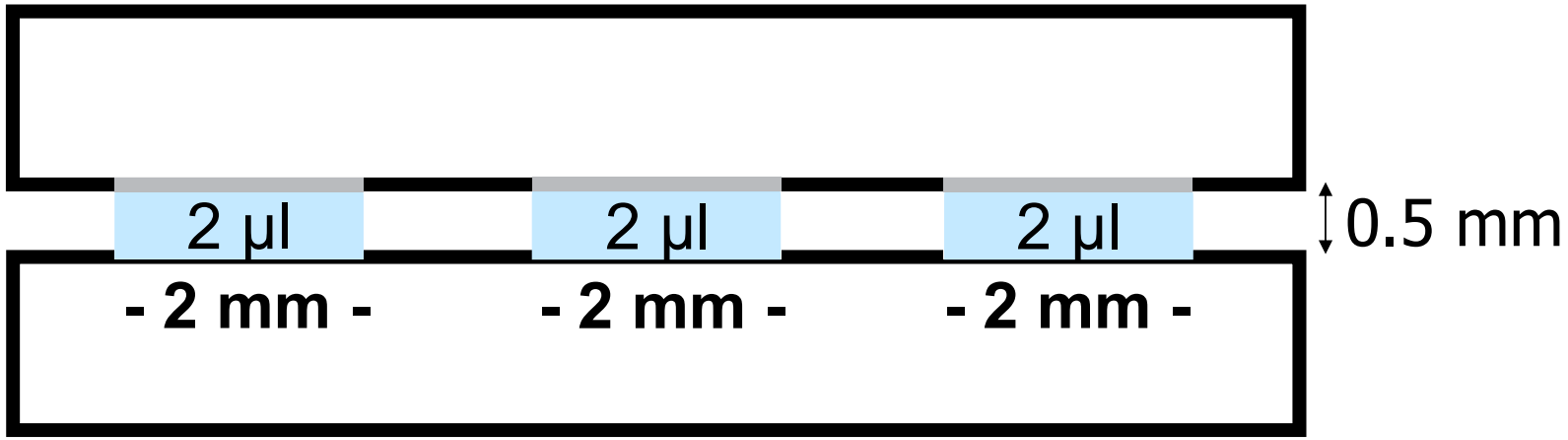
氣體控制器


電源
濃度計
CO_2開關
O_2開關
濃度計
POWER
CO_2
CO_2 GAS
ALARM BUZZER
CO_2 OUT OF RANGE
FAN ALARM
PUMP ALARM
O_2 OUT OF RANGE
O_2 GAS
O_2
CO_2/ O_2 Gas Controller
BioTek


連接flow meter上方的
接孔與Gas controller
連接flow meter下方的接孔與鋼瓶
N_2 鋼瓶和壓力錶
N_2
連接flow meter上方的
接孔與Gas controller
連接flow meter下方的接孔與鋼瓶
CO_2
CO_2 鋼瓶和壓力錶
Gas Infusion Tubing Set (2 shown)
with 5 micron particulate filter
BTI PN 1213023
Filters
透明
白色
黃色
黑色
Hydrophobic filter assembly
0.2 micron filter
BTI PN 1210505
Mixing Fan Power Cable
(instrument-specific)

Take3 超微量定量偵測盤

Take3 超微量定量偵測盤



應用

- ELISA
- Toxicity & drug screening
- Protein quantitation
- Nucleic acid quantitation
- Chemical measurements
- Cell viability, proliferation & death
- Gene expression

儀器規格

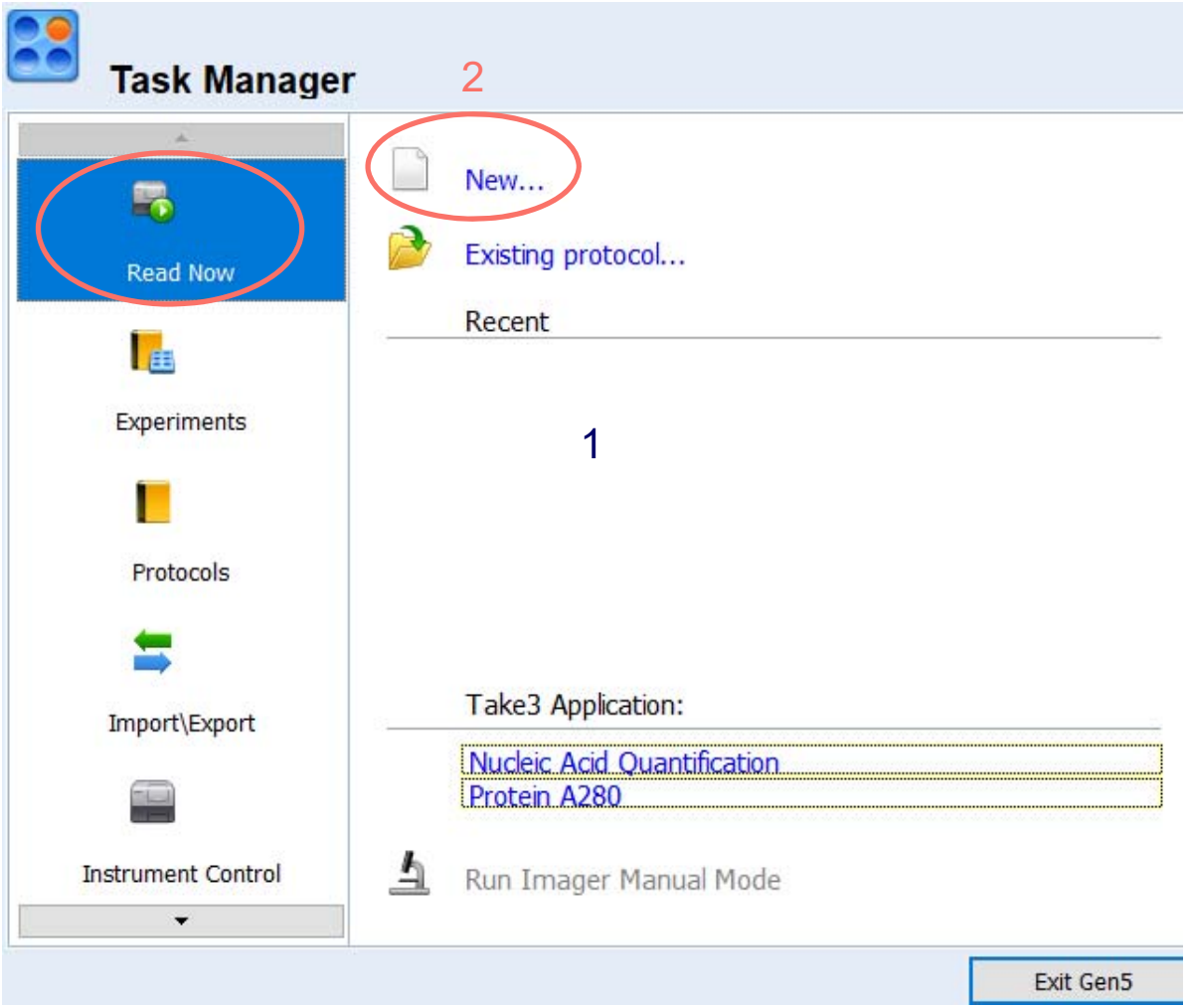
- 吸收光: 230 - 999 nm
- 螢冷光: 濾鏡式 或 全波長式 250-700nm
- 0 - 4.0 OD
- 溫度控制
- 震盪功能

Gen5 分析軟體

Gen5 軟體功能

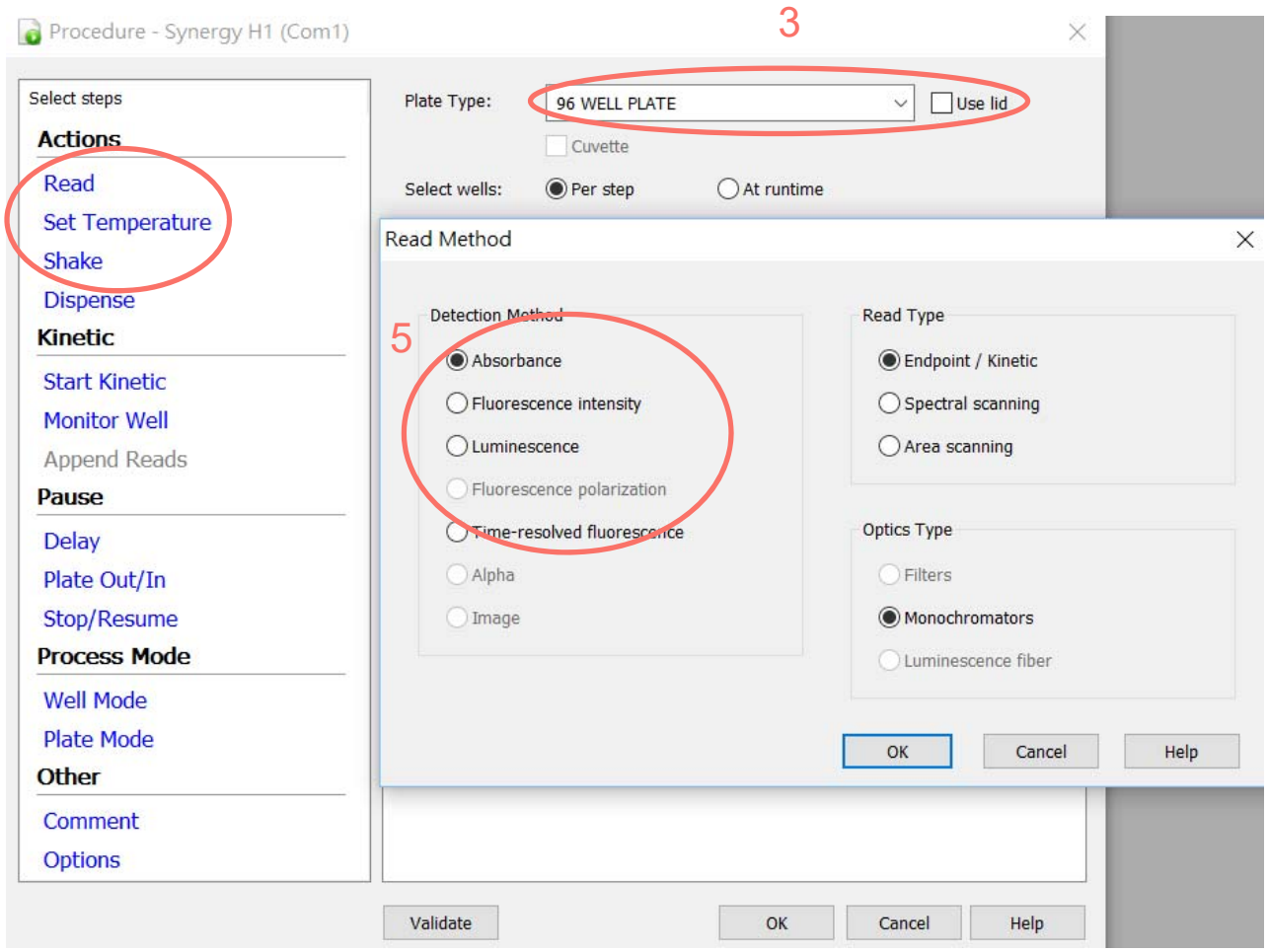
- 盤型
- 設定波長、溫度、震盪
- Plate Layout: 樣本佈局
- Data Reduction: 讀值運算及標準曲線
- 匯出報告

Gen5 軟體功能

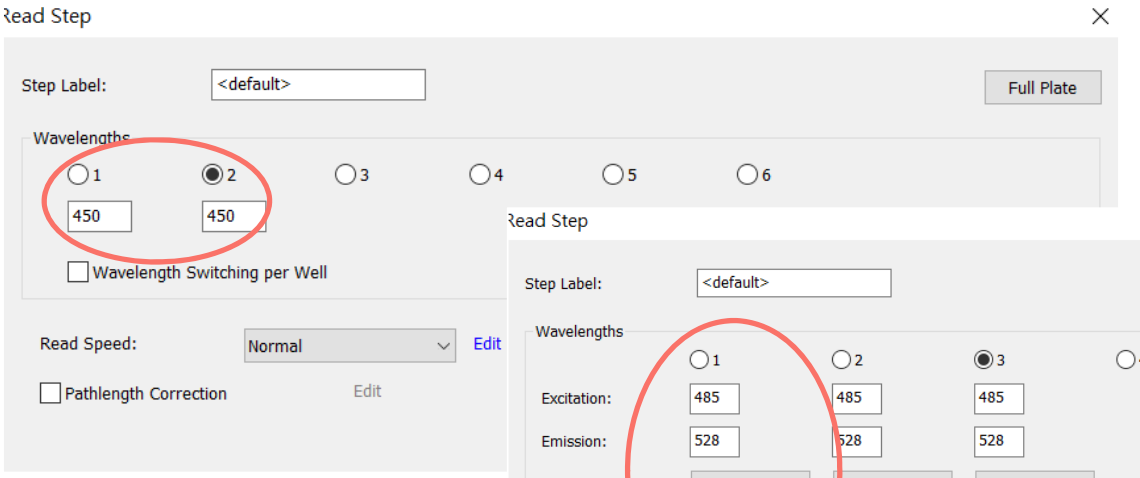


- Experiment
- Protocol

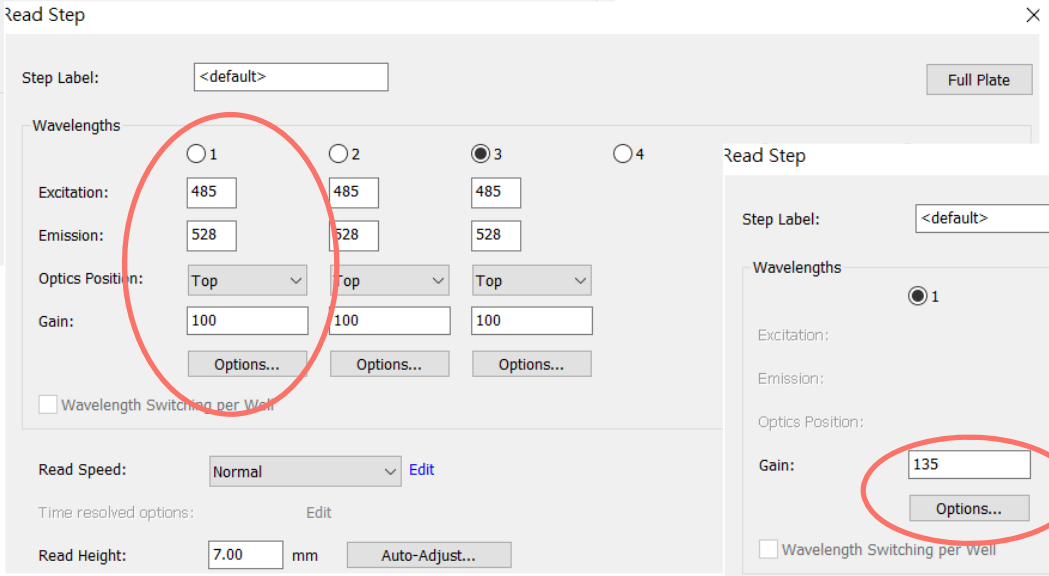
Gen5 軟體功能


3
Procedure - Synergy H1 (Com1)
Select steps
Actions
Read
Set Temperature
Shake
Dispense
Kinetic
Start Kinetic
Monitor Well
Append Reads
Pause
Delay
Plate Out/In
Stop/Resume
Process Mode
Well Mode
Plate Mode
Other
Comment
Options
4
Plate Type:
96 WELL PLATE
Use lid
Cuvette
Select wells:
Per step
At runtime
Read Method
5
Detection Method
Absorbance
Fluorescence intensity
Luminescence
Fluorescence polarization
Time-resolved fluorescence
Alpha
Image
Read Type
Endpoint / Kinetic
Spectral scanning
Area scanning
Optics Type
Filters
Monochromators
Luminescence fiber
OK
Cancel
Help
Validate
OK
Cancel
Help

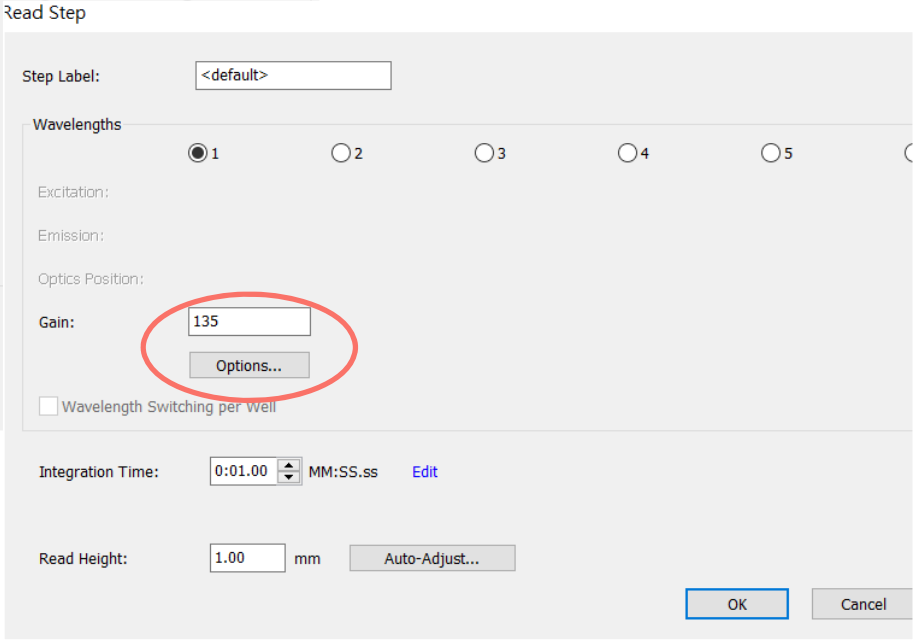
Gen5 軟體功能



吸收光

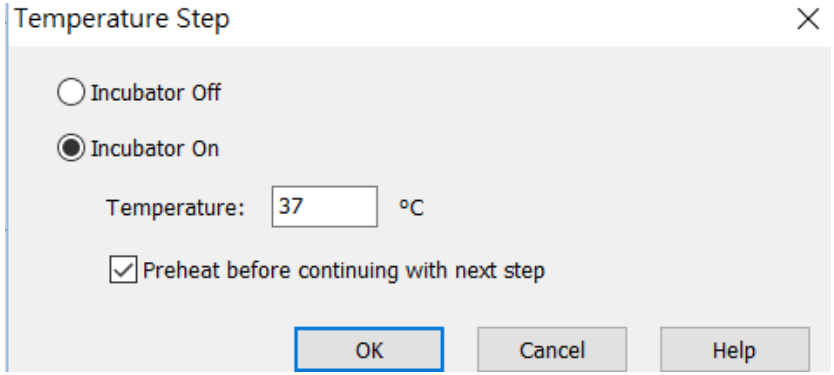


螢光

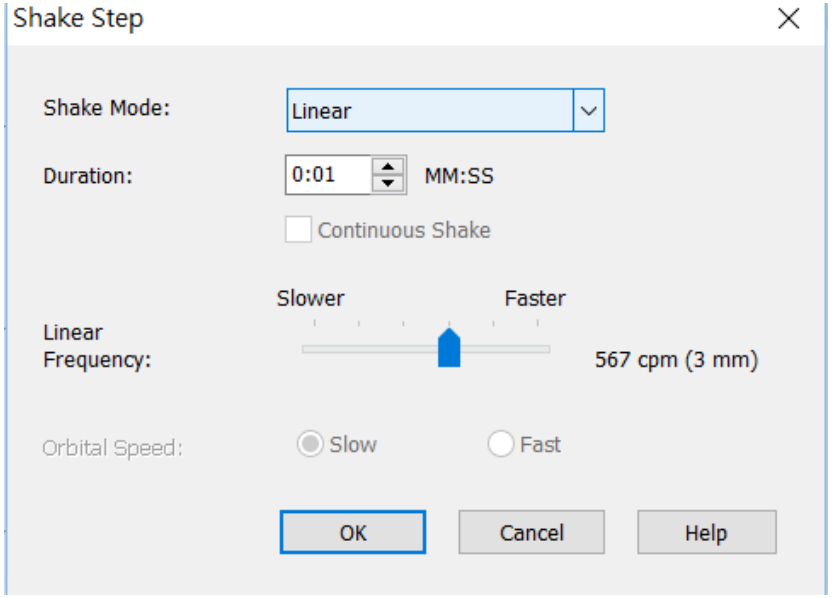


冷光

Gen5 軟體功能



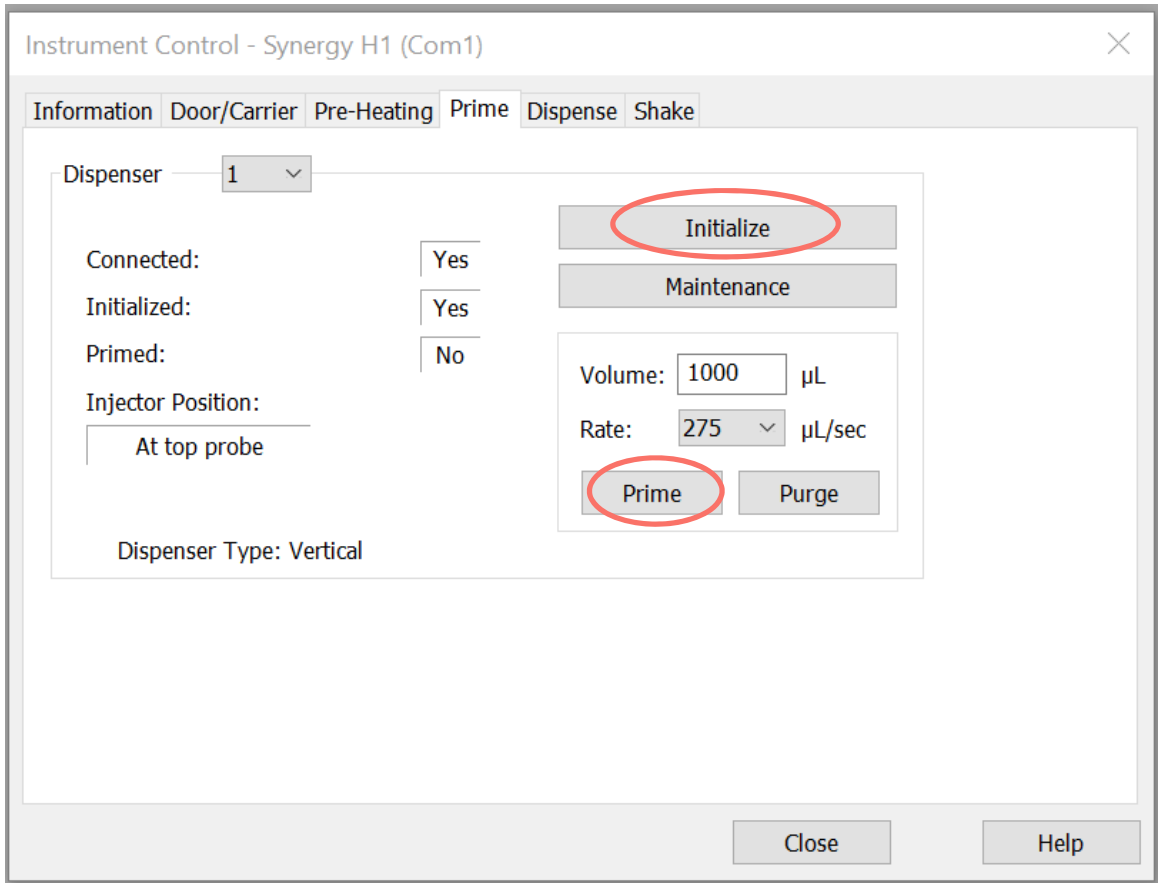
設定溫度



設定震盪

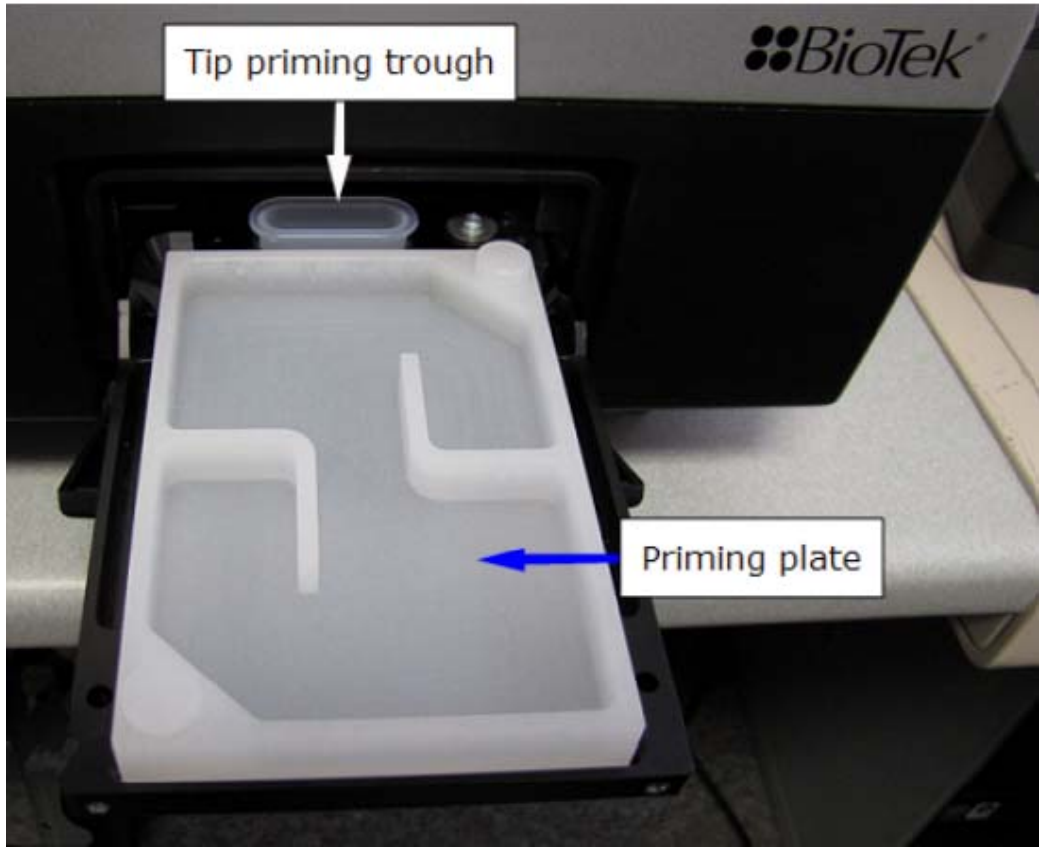
Gen5 軟體功能 Dispense Module Control

設定步驟之前到 System > Instrument Control > Synergy H1>Initialize

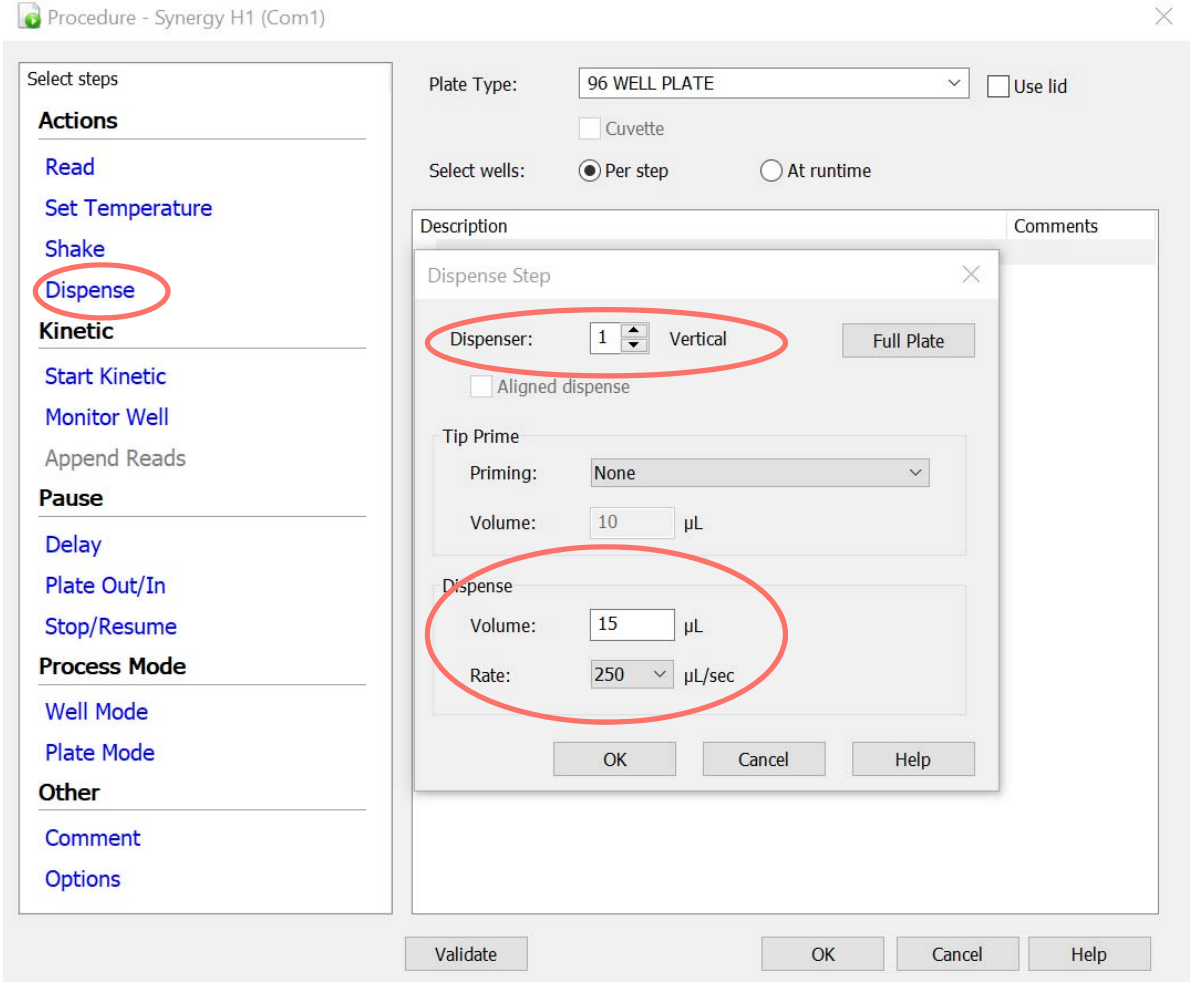


Gen5 軟體功能 Dispense Module Control

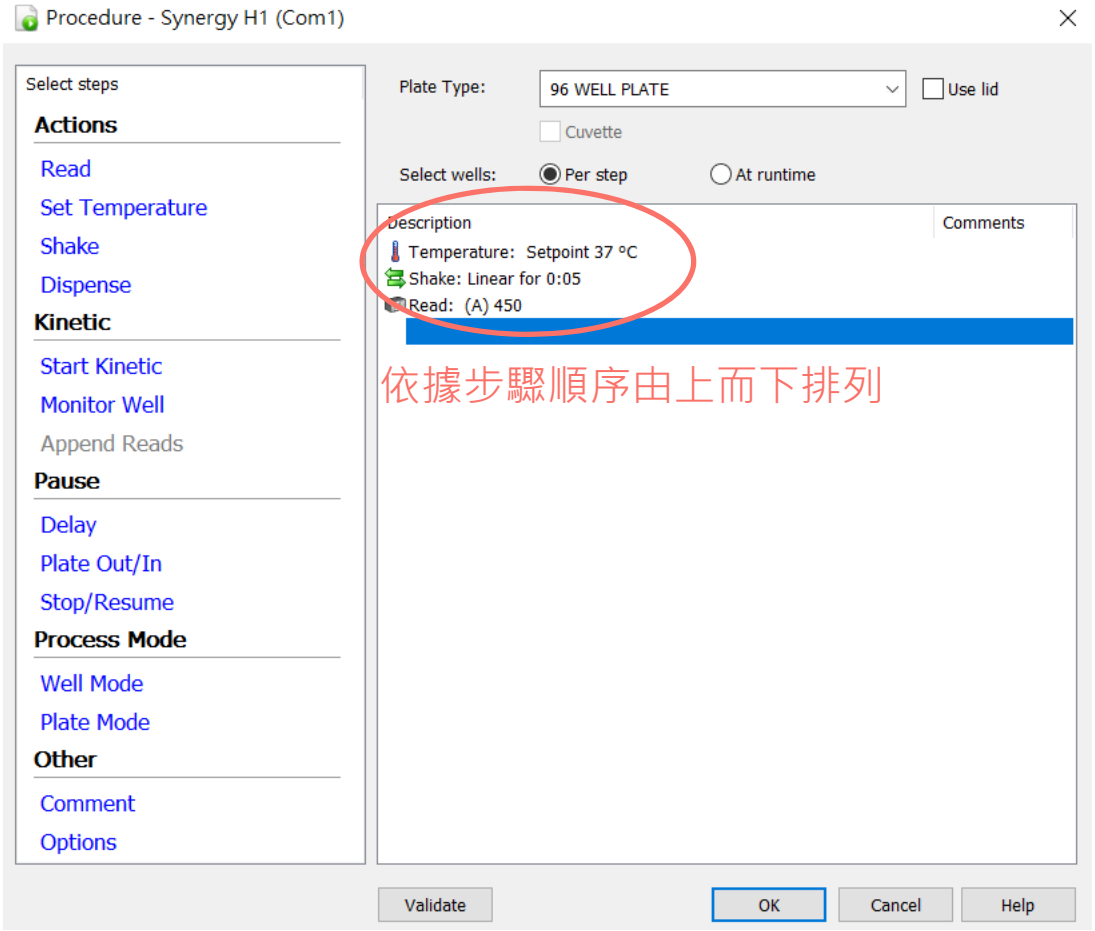
按下 Prime 之前先放好 Tip priming trough 和 Priming Plate



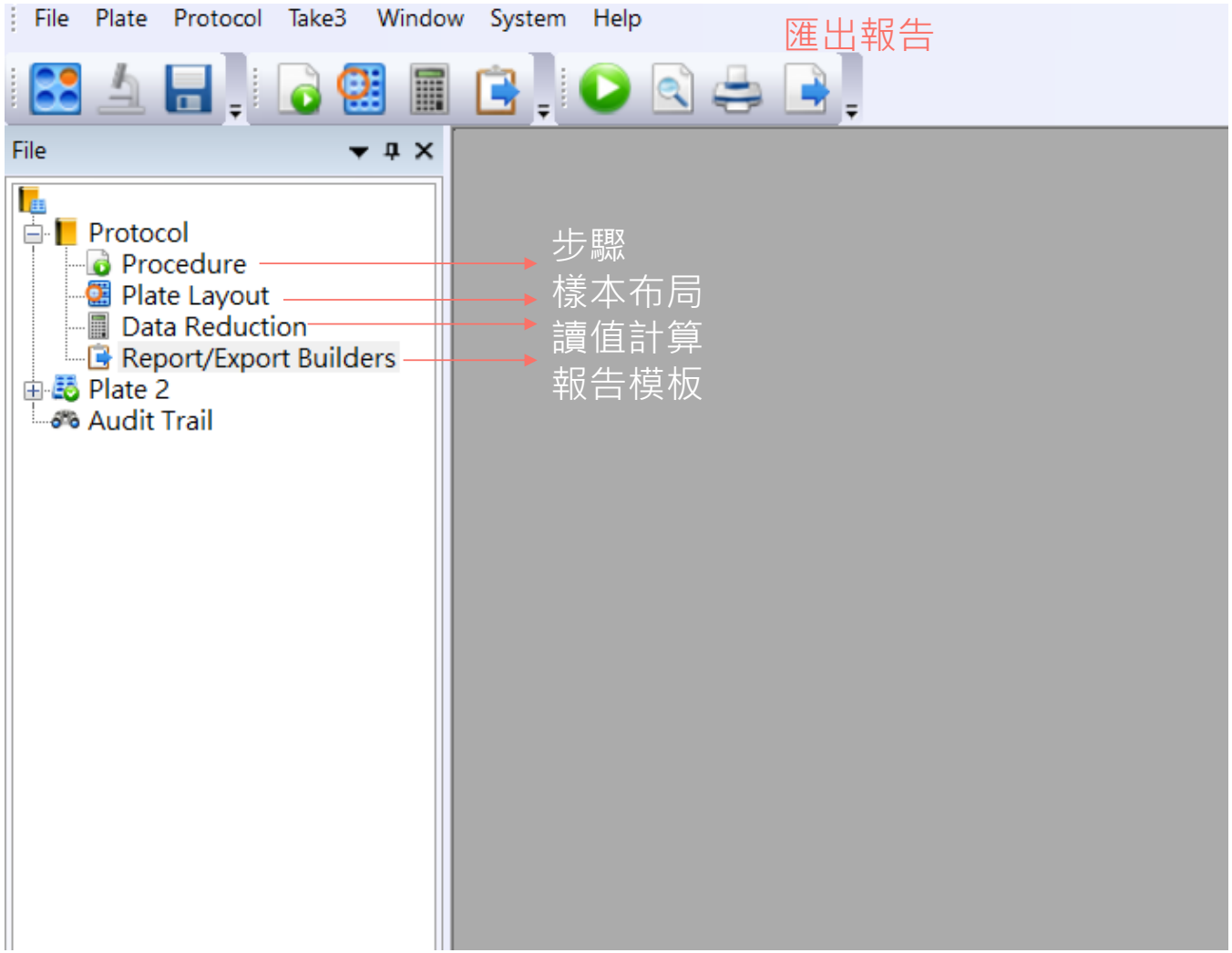
Gen5 軟體功能 Dispense Module Control



Gen5 軟體功能


Procedure - Synergy H1 (Com1)
Select steps
Actions
Read
Set Temperature
Shake
Dispense
Kinetic
Start Kinetic
Monitor Well
Append Reads
Pause
Delay
Plate Out/In
Stop/Resume
Process Mode
Well Mode
Plate Mode
Other
Comment
Options
Plate Type:
96 WELL PLATE
Use lid
Cuvette
Select wells:
Per step
At runtime
Description
Comments
Temperature: Setpoint 37 °C
Shake: Linear for 0:05
Read: (A) 450
依據步驟順序由上而下排列
Validate
OK
Cancel
Help

Gen5 軟體功能


File
Plate
Protocol
Take3
Window
System
Help
匯出報告
File
Protocol
Procedure
Plate Layout
Data Reduction
Report/Export Builders
Plate 2
Audit Trail
步驟
樣本布局
讀值計算
報告模板

Gen5 軟體功能

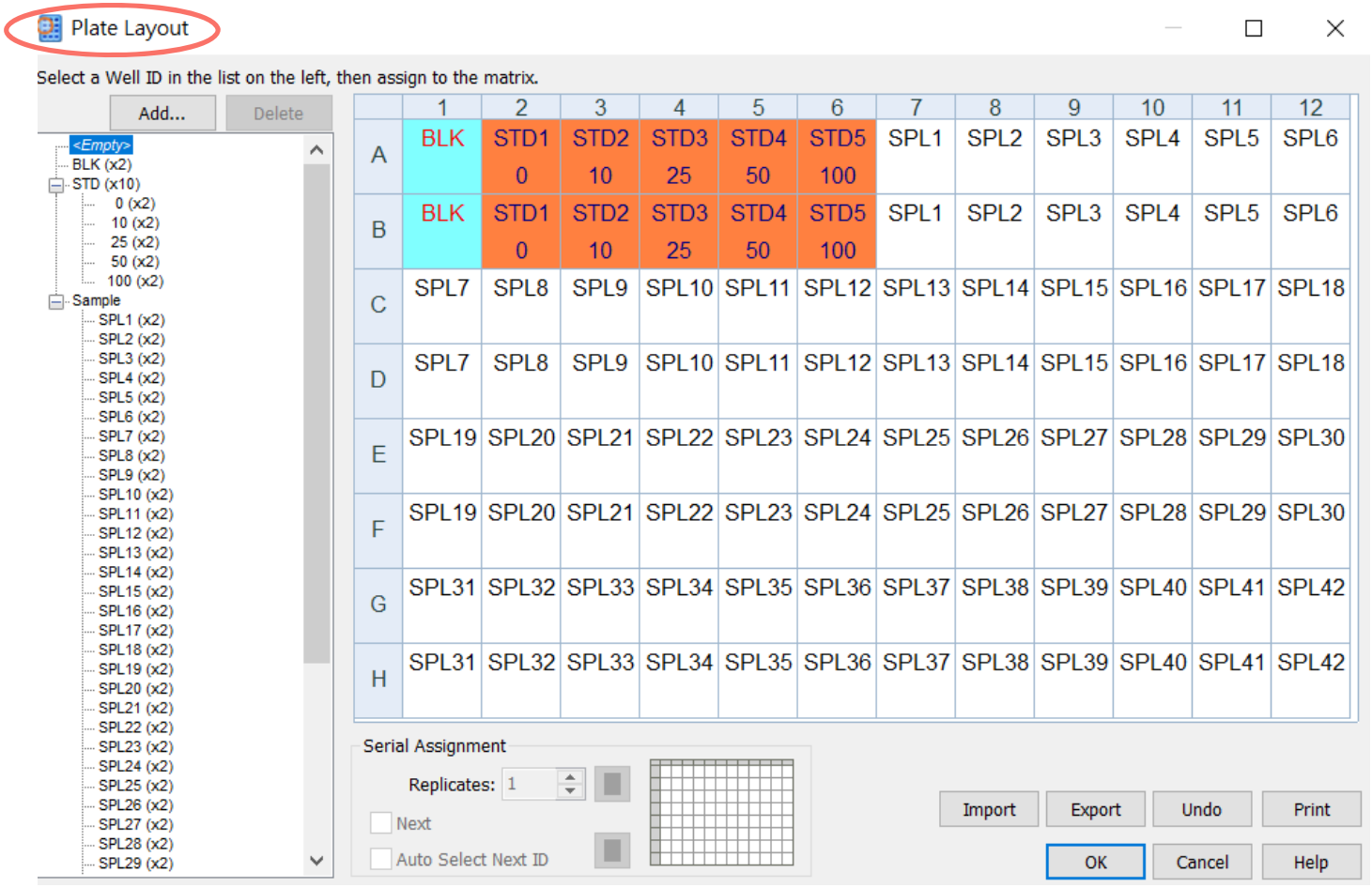
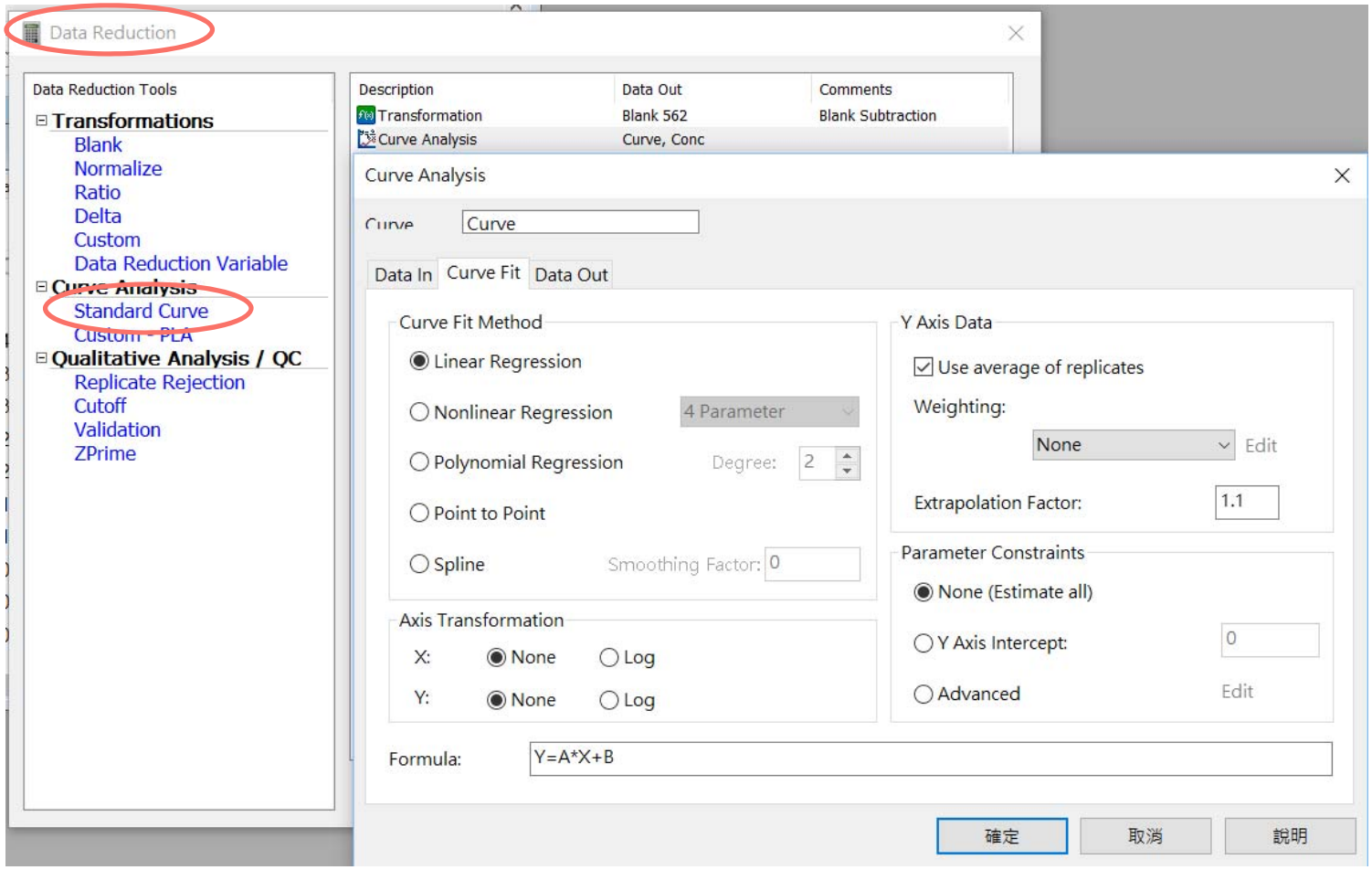
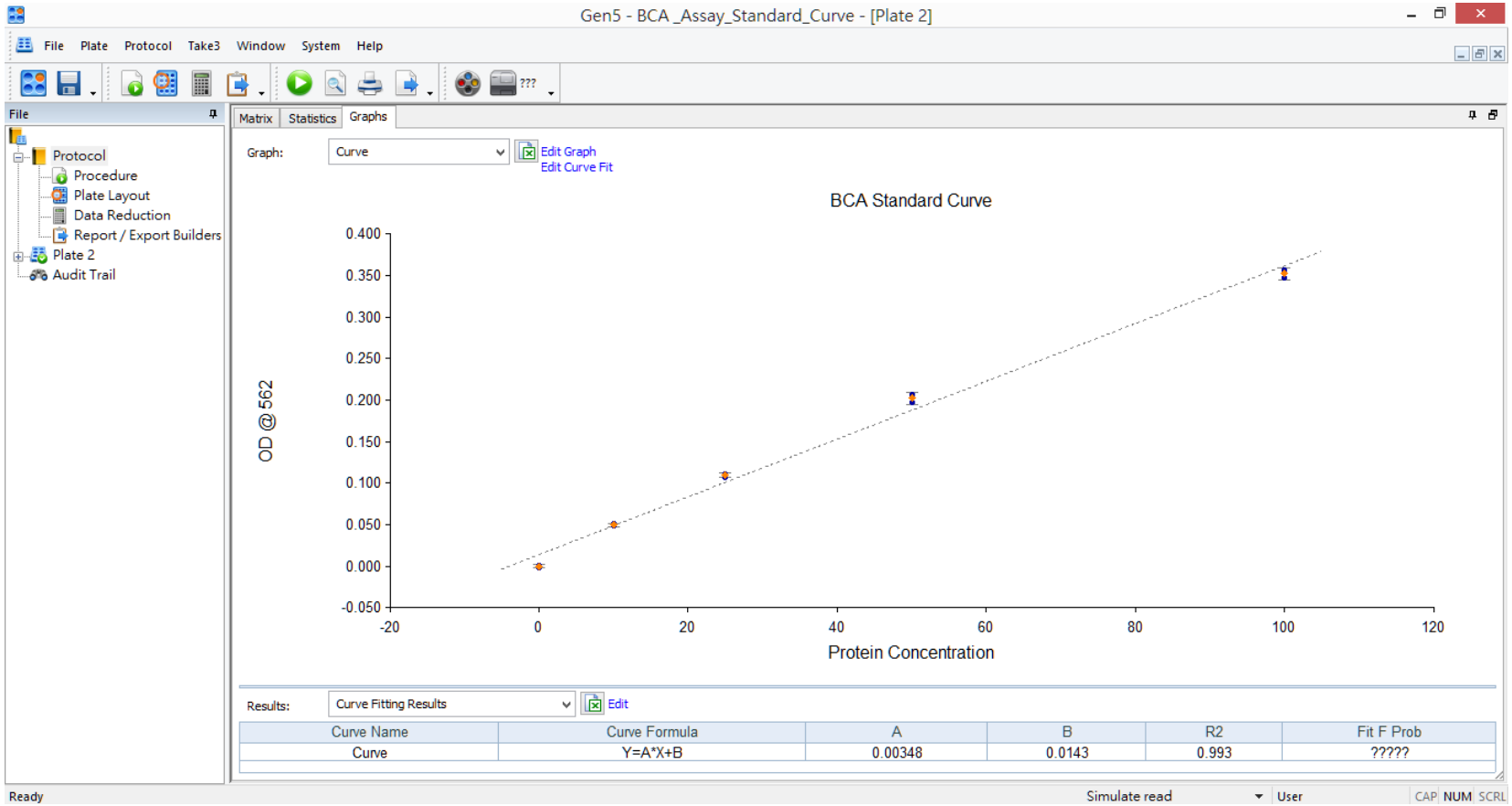


Plate Layout
Select a Well ID in the list on the left, then assign to the matrix.
Add...
Delete
<Empty>
BLK (x2)
STD (x10)
0 (x2)
10 (x2)
25 (x2)
50 (x2)
100 (x2)
Sample
SPL1 (x2)
SPL2 (x2)
SPL3 (x2)
SPL4 (x2)
SPL5 (x2)
SPL6 (x2)
SPL7 (x2)
SPL8 (x2)
SPL9 (x2)
SPL10 (x2)
SPL11 (x2)
SPL12 (x2)
SPL13 (x2)
SPL14 (x2)
SPL15 (x2)
SPL16 (x2)
SPL17 (x2)
SPL18 (x2)
SPL19 (x2)
SPL20 (x2)
SPL21 (x2)
SPL22 (x2)
SPL23 (x2)
SPL24 (x2)
SPL25 (x2)
SPL26 (x2)
SPL27 (x2)
SPL28 (x2)
SPL29 (x2)
1 2 3 4 5 6 7 8 9 10 11 12
A BLK STD1 0 STD2 10 STD3 25 STD4 50 STD5 100 SPL1 SPL2 SPL3 SPL4 SPL5 SPL6
B BLK STD1 0 STD2 10 STD3 25 STD4 50 STD5 100 SPL1 SPL2 SPL3 SPL4 SPL5 SPL6
C SPL7 SPL8 SPL9 SPL10 SPL11 SPL12 SPL13 SPL14 SPL15 SPL16 SPL17 SPL18
D SPL7 SPL8 SPL9 SPL10 SPL11 SPL12 SPL13 SPL14 SPL15 SPL16 SPL17 SPL18
E SPL19 SPL20 SPL21 SPL22 SPL23 SPL24 SPL25 SPL26 SPL27 SPL28 SPL29 SPL30
F SPL19 SPL20 SPL21 SPL22 SPL23 SPL24 SPL25 SPL26 SPL27 SPL28 SPL29 SPL30
G SPL31 SPL32 SPL33 SPL34 SPL35 SPL36 SPL37 SPL38 SPL39 SPL40 SPL41 SPL42
H SPL31 SPL32 SPL33 SPL34 SPL35 SPL36 SPL37 SPL38 SPL39 SPL40 SPL41 SPL42
Serial Assignment
Replicates: 1
Next
Auto Select Next ID
Import
Export
Undo
Print
OK
Cancel
Help

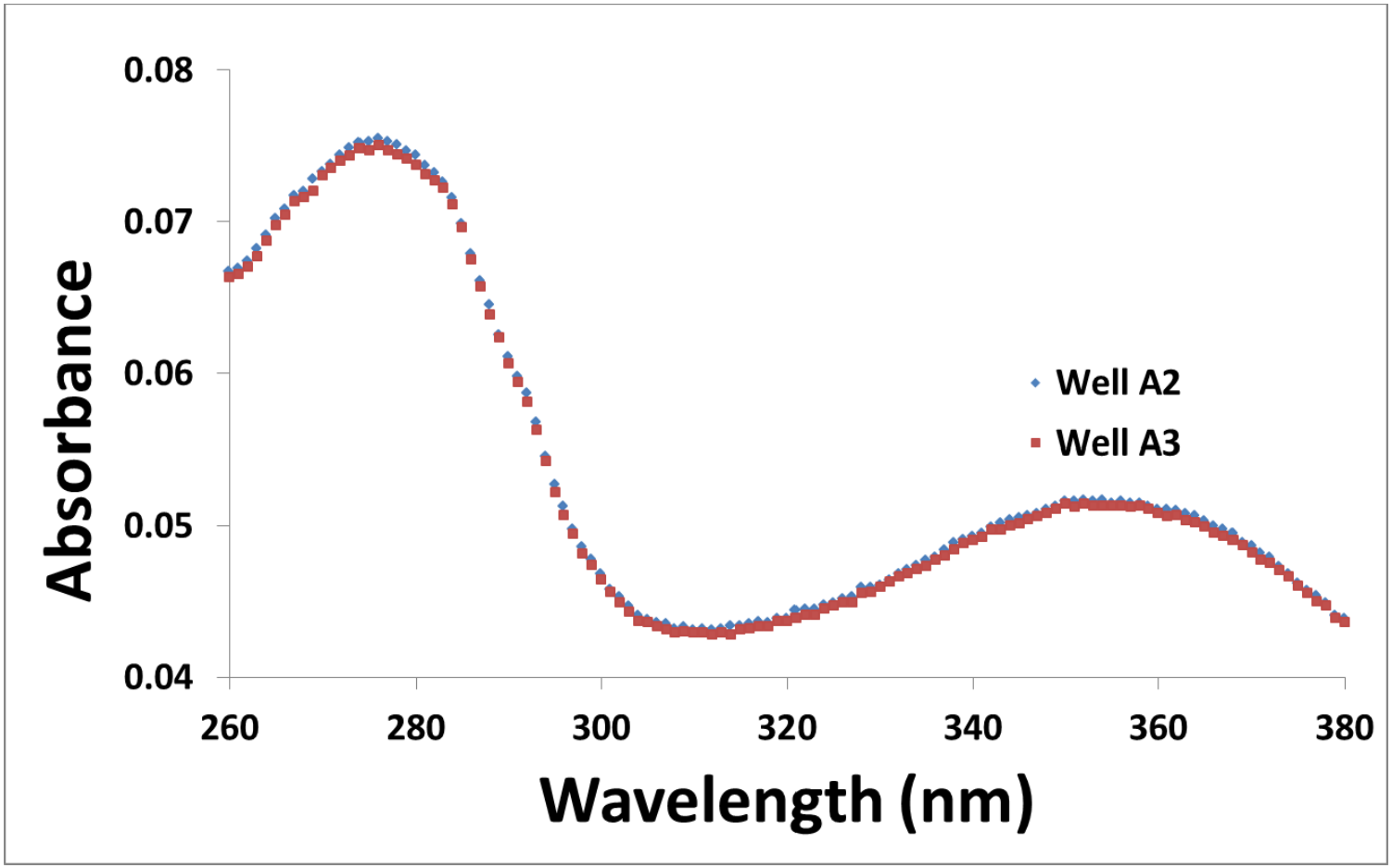
Gen5 軟體功能


Data Reduction
Data Reduction Tools
Transformations
Blank
Normalize
Ratio
Delta
Custom
Data Reduction Variable
Curve Analysis
Standard Curve
Custom - PLA
Qualitative Analysis / QC
Replicate Rejection
Cutoff
Validation
ZPrime
Description
Data Out
Comments
Transformation
Blank 562
Blank Subtraction
Curve Analysis
Curve, Conc
Curve Analysis
Curve
Curve
Data In
Curve Fit
Data Out
Curve Fit Method
Linear Regression
Nonlinear Regression
4 Parameter
Polynomial Regression
Degree:
2
Point to Point
Spline
Smoothing Factor:
0
Axis Transformation
X:
None
Log
Y:
None
Log
Y Axis Data
Use average of replicates
Weighting:
None
Edit
Extrapolation Factor:
1.1
Parameter Constraints
None (Estimate all)
Y Axis Intercept:
0
Advanced
Edit
Formula:
Y=A*X+B
確定
取消
說明

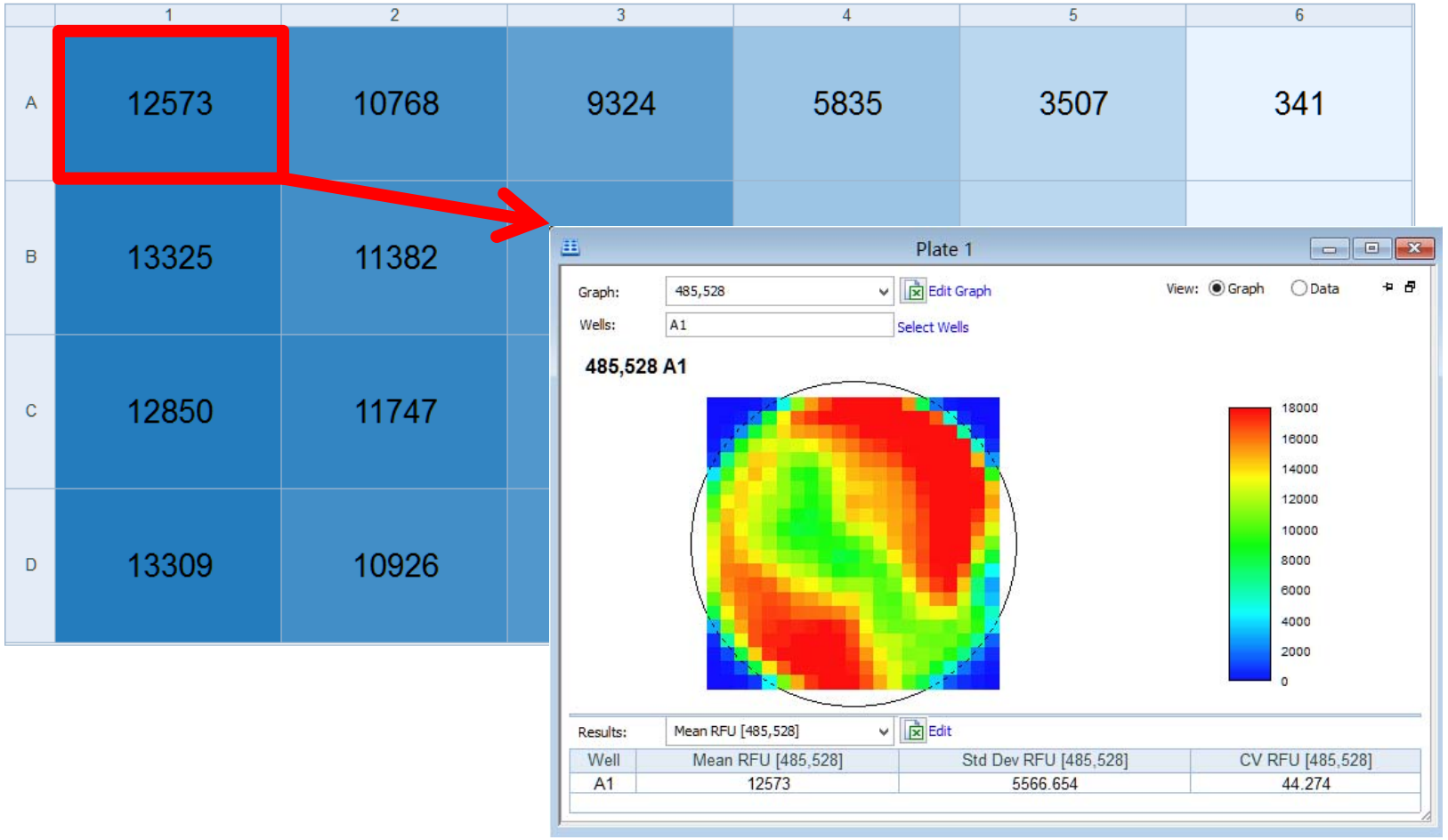
Gen5 軟體功能


Gen5 - BCA _Assay_Standard_Curve - [Plate 2]
File Plate Protocol Take3 Window System Help
File
Protocol
Procedure
Plate Layout
Data Reduction
Report / Export Builders
Plate 2
Audit Trail
Matrix Statistics Graphs
Graph: Curve
Edit Graph
Edit Curve Fit
BCA Standard Curve
OD @ 562
0.400
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
-0.050
-20 0 20 40 60 80 100 120
Protein Concentration
Results: Curve Fitting Results
Edit
Curve Name | Curve Formula | A | B | R2 | Fit F Prob
Curve | Y=A*X+B | 0.00348 | 0.0143 | 0.993 | ?????
Ready
Simulate read
User
CAP NUM SCRL

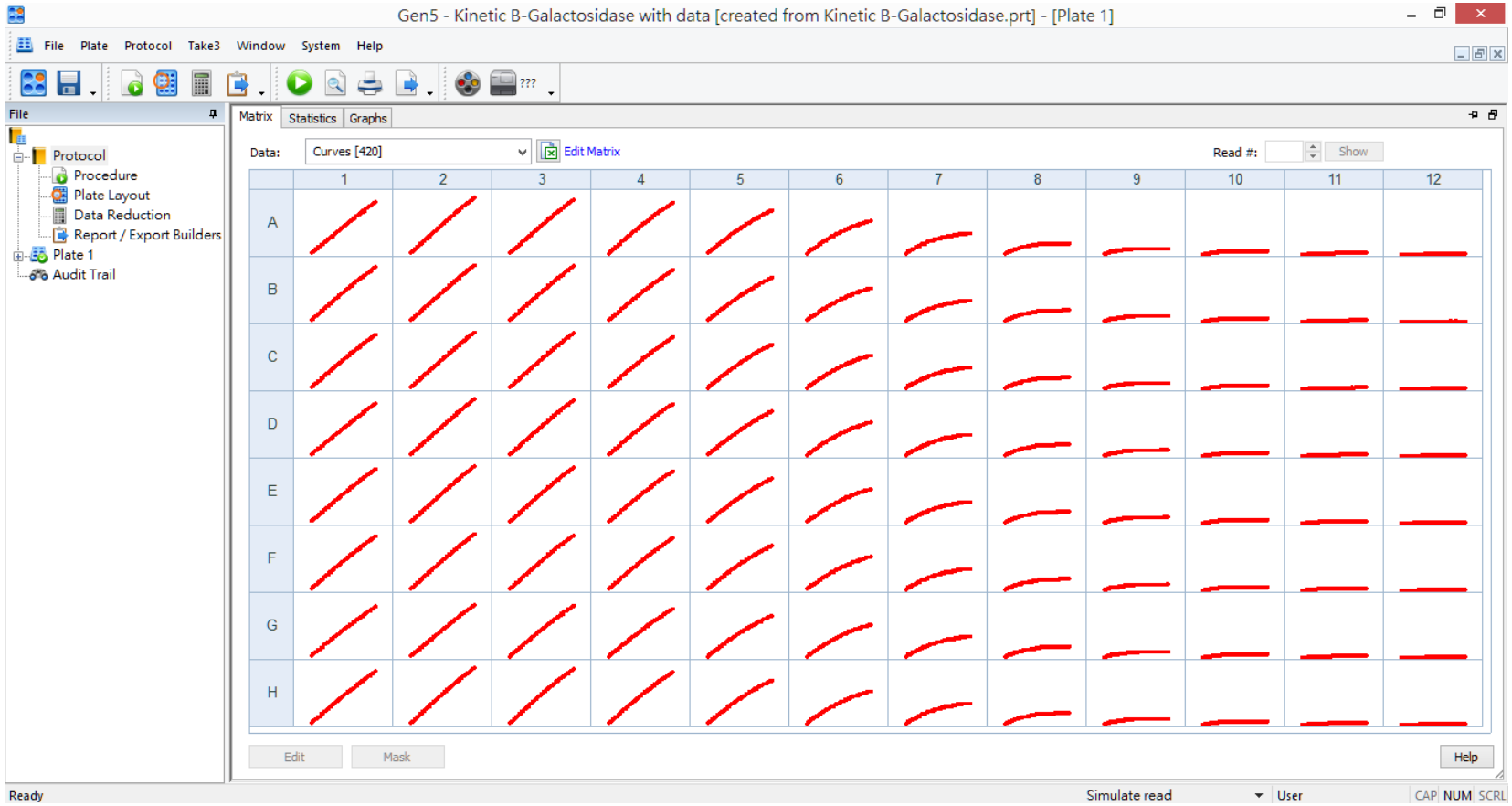
Gen5 軟體功能: 光譜掃描



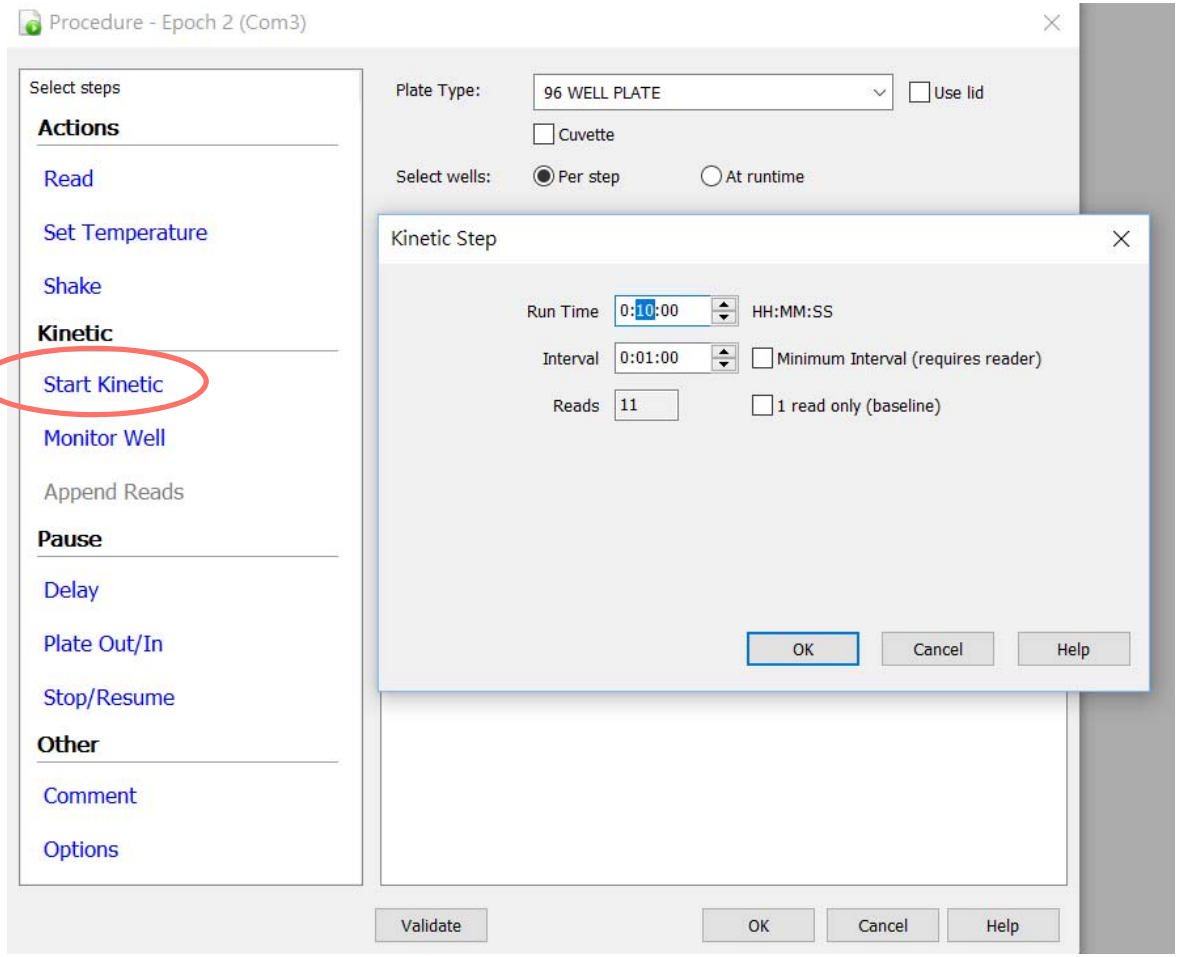
Gen5 軟體功能: 孔域掃描



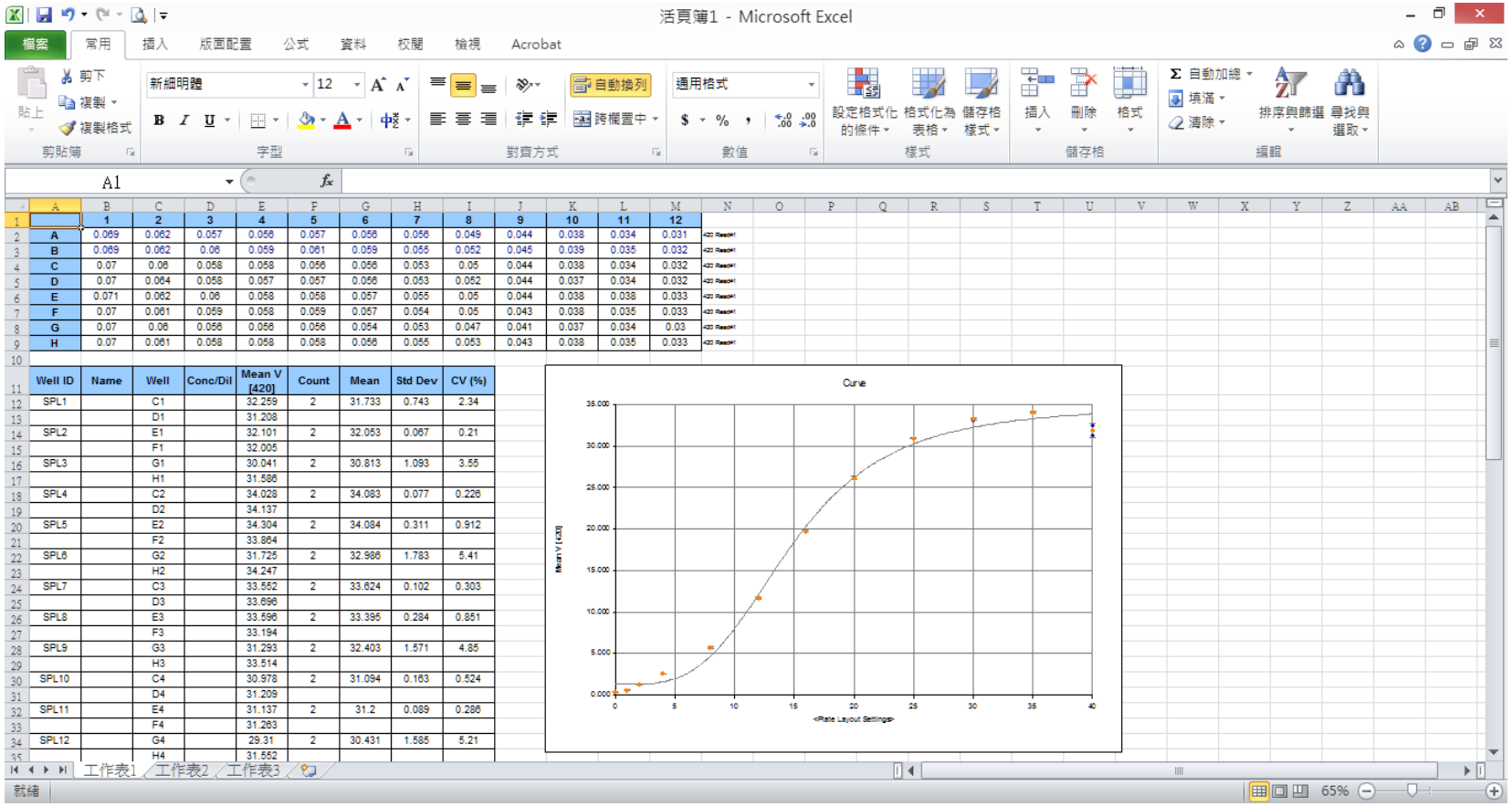
Gen5 軟體功能: Kinetic



Gen5 軟體功能: Kinetic



Gen5 軟體功能: 匯出報告

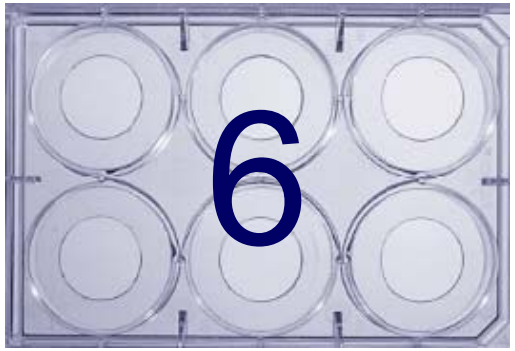


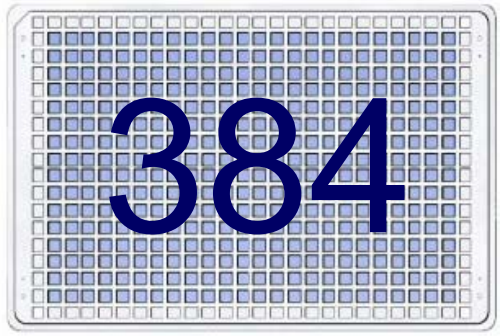
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.069	0.062	0.057	0.056	0.057	0.056	0.056	0.049	0.044	0.038	0.034	0.031
B	0.069	0.062	0.06	0.059	0.061	0.059	0.055	0.052	0.045	0.039	0.035	0.032
C	0.07	0.06	0.058	0.058	0.056	0.056	0.053	0.05	0.044	0.038	0.034	0.032
D	0.07	0.064	0.058	0.057	0.057	0.056	0.053	0.052	0.044	0.037	0.034	0.032
E	0.071	0.062	0.06	0.058	0.058	0.057	0.055	0.05	0.044	0.038	0.038	0.033
F	0.07	0.061	0.059	0.058	0.059	0.057	0.054	0.05	0.043	0.038	0.035	0.033
G	0.07	0.06	0.056	0.056	0.056	0.054	0.053	0.047	0.041	0.037	0.034	0.03
H	0.07	0.061	0.058	0.058	0.058	0.056	0.055	0.053	0.043	0.038	0.035	0.033

Well ID	Name	Well	Conc/Dil	Mean V [420]	Count	Mean	Std Dev	CV (%)
SPL1		C1		32.259	2	31.733	0.743	2.34
		D1		31.208				
SPL2		E1		32.101	2	32.053	0.067	0.21
		F1		32.005				
SPL3		G1		30.041	2	30.813	1.093	3.55
		H1		31.586				
SPL4		C2		34.028	2	34.083	0.077	0.226
		D2		34.137				
SPL5		E2		34.304	2	34.084	0.311	0.912
		F2		33.864				
SPL6		G2		31.725	2	32.986	1.783	5.41
		H2		34.247				
SPL7		C3		33.552	2	33.624	0.102	0.303
		D3		33.696				
SPL8		E3		33.596	2	33.395	0.284	0.851
		F3		33.194				
SPL9		G3		31.293	2	32.403	1.571	4.85
		H3		33.514				
SPL10		C4		30.978	2	31.094	0.163	0.524
		D4		31.209				
SPL11		E4		31.137	2	31.2	0.089	0.286
		F4		31.263				
SPL12		G4		29.31	2	30.431	1.585	5.21
		H4		31.552				

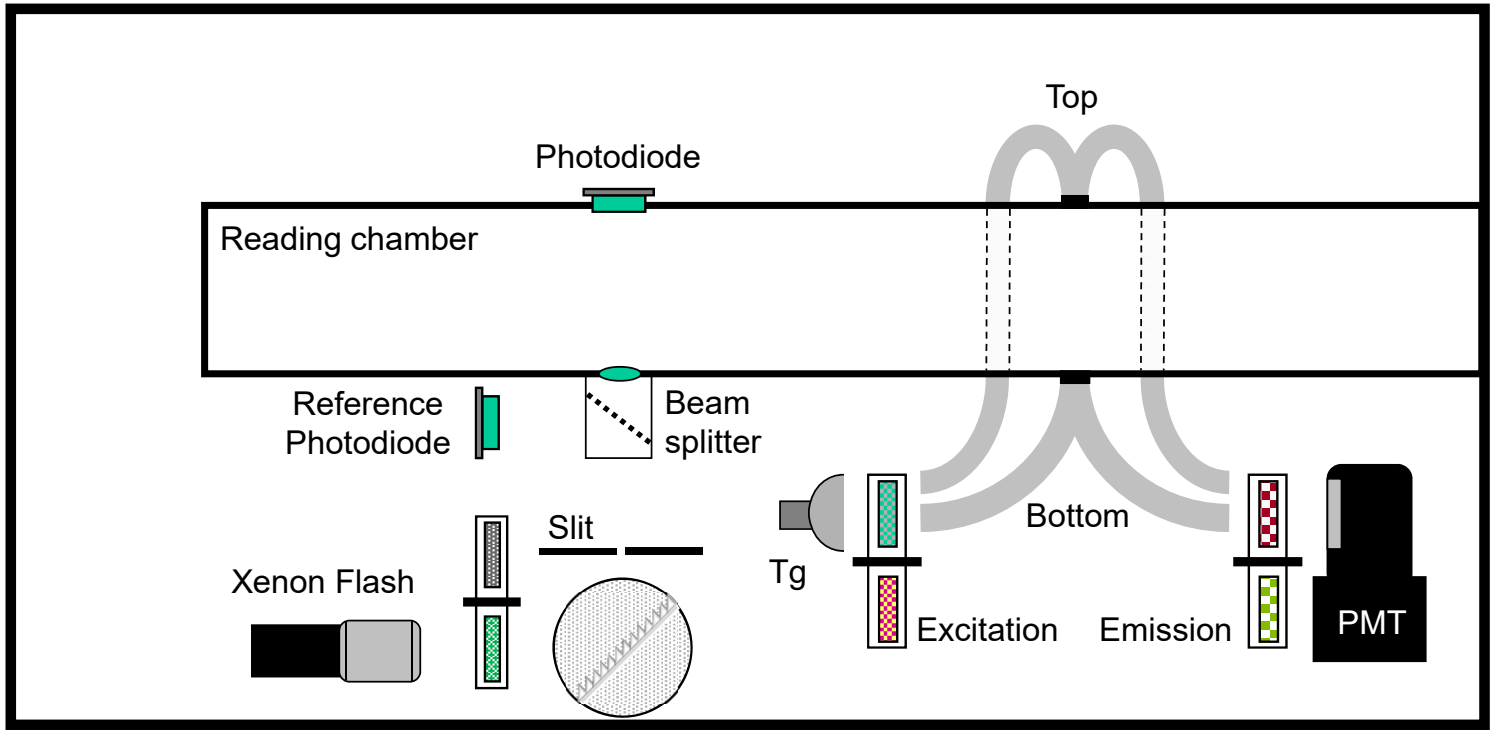
Synergy HTX

微量盤類型

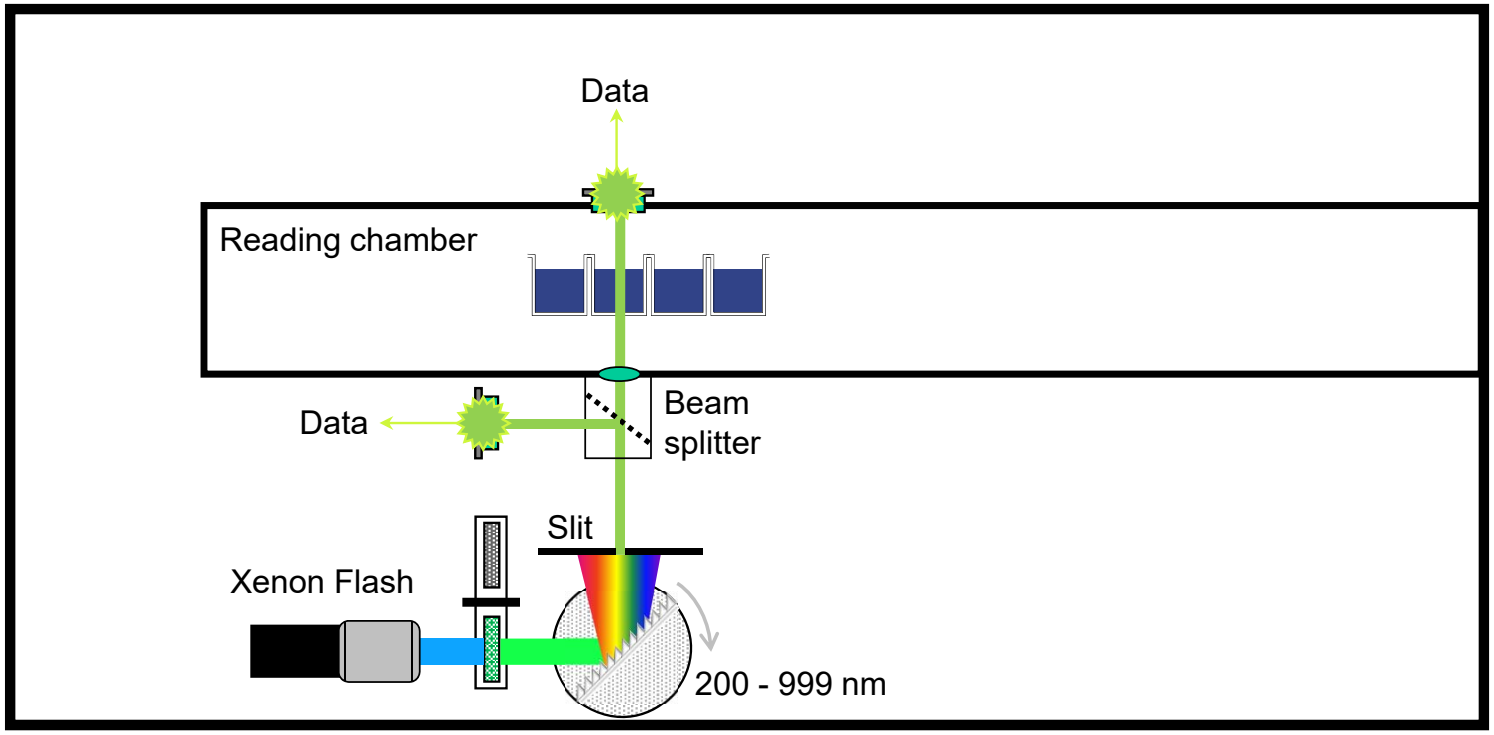




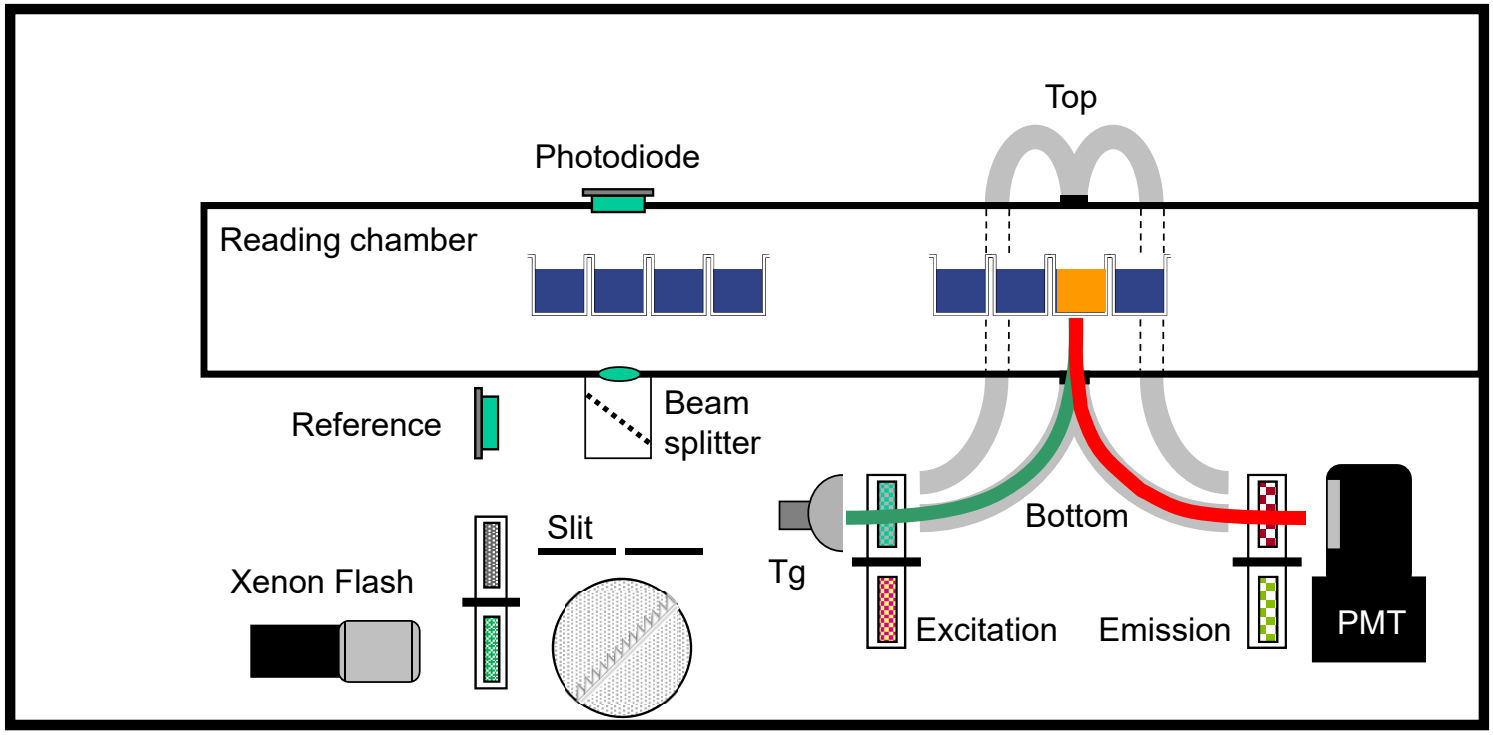
偵測模組


Top
Photodiode
Reading chamber
Reference
Photodiode
Beam
splitter
Slit
Xenon Flash
Tg
Bottom
Excitation
Emission
PMT

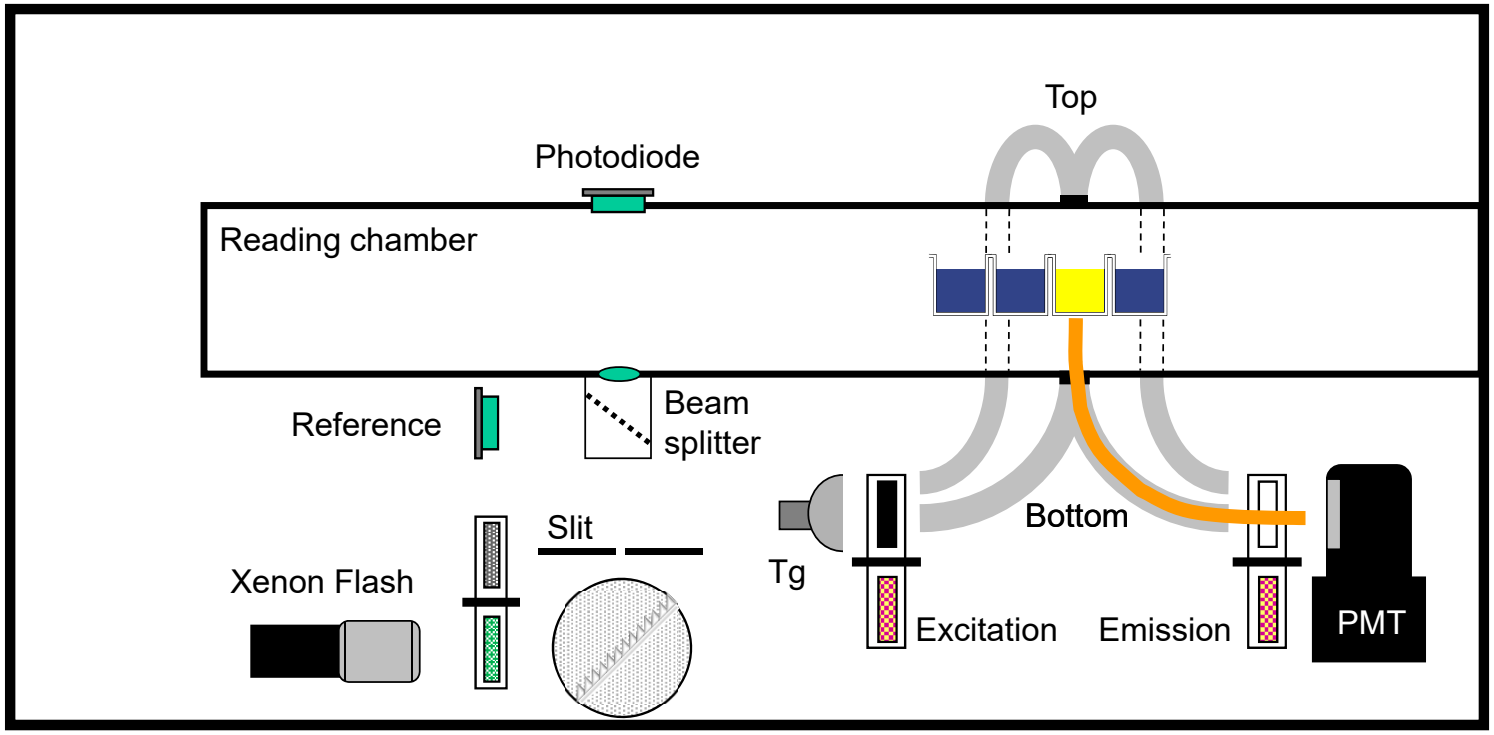
吸收光偵測路徑


Data
Reading chamber
Data
Beam
splitter
Slit
Xenon Flash
200 - 999 nm

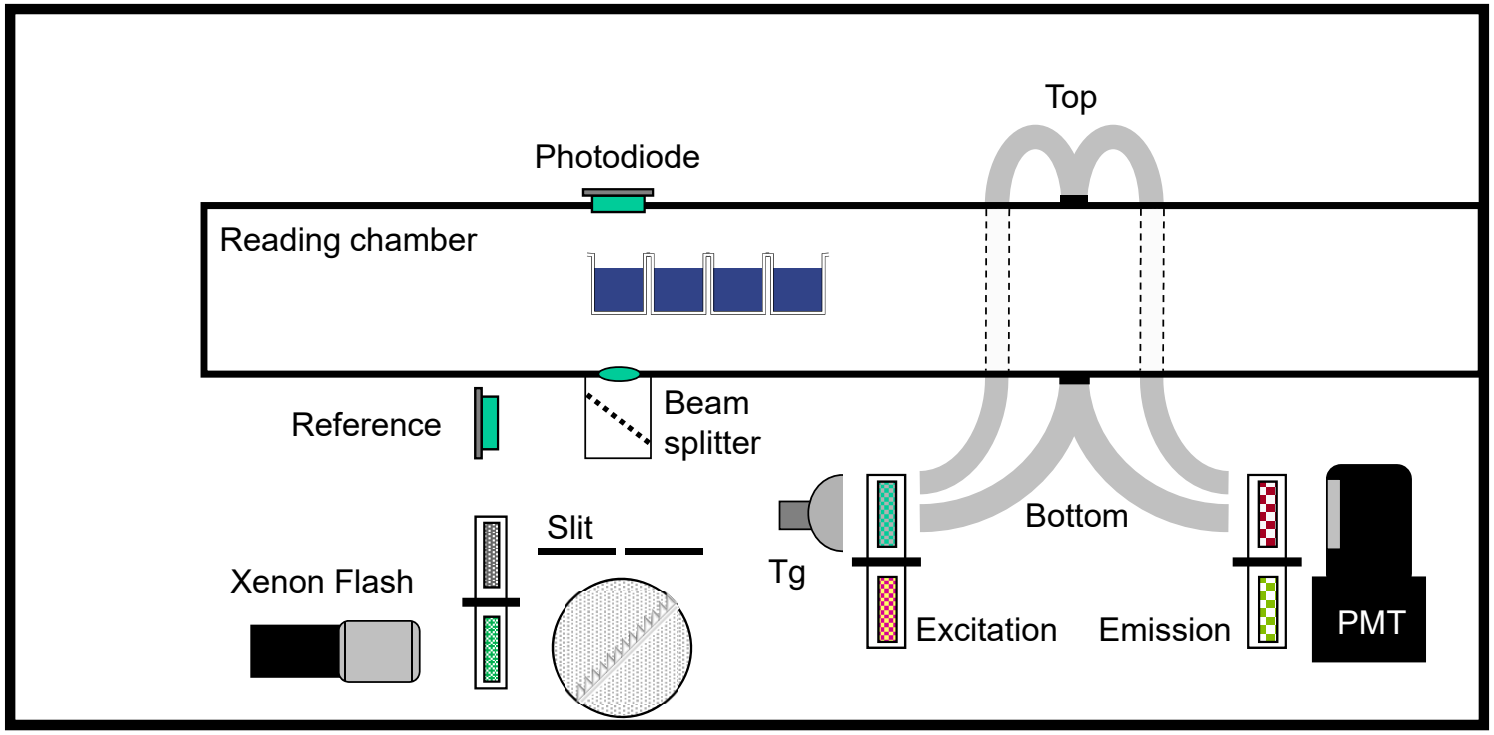
螢光偵測路徑


Top
Photodiode
Reading chamber
Reference
Beam
splitter
Slit
Xenon Flash
Tg
Bottom
Excitation
Emission
PMT

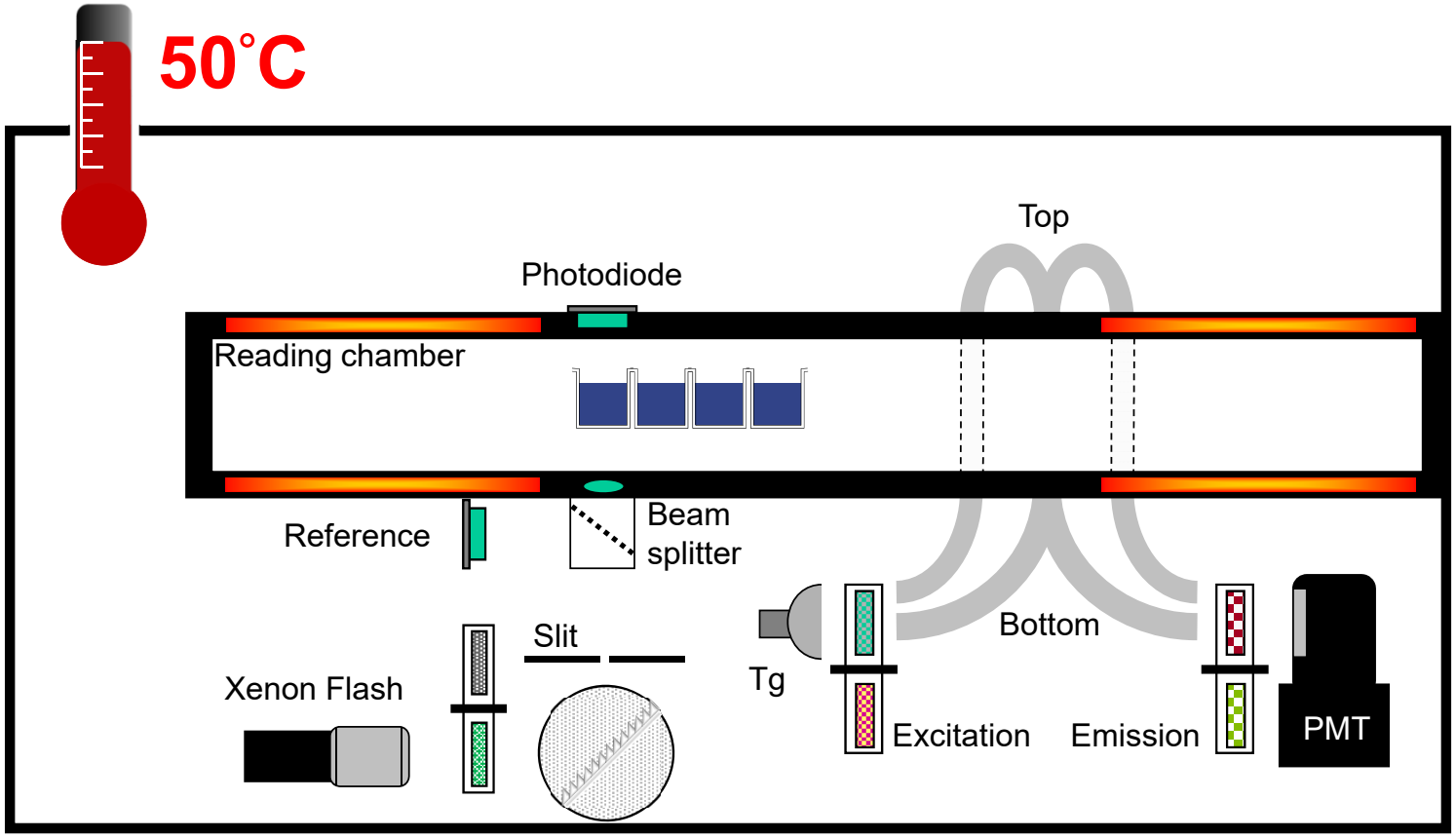
冷光偵測路徑

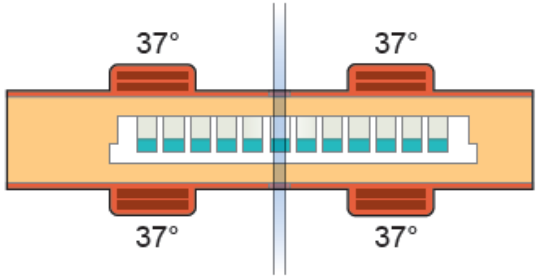

Top
Photodiode
Reading chamber
Reference
Beam
splitter
Slit
Xenon Flash
Tg
Bottom
Excitation
Emission
PMT

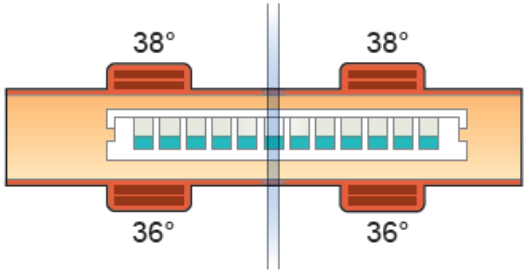
震盪功能


Top
Photodiode
Reading chamber
Reference
Beam
splitter
Slit
Xenon Flash
Tg
Bottom
Excitation
Emission
PMT

溫度控制: 溫度梯度防止水氣凝結







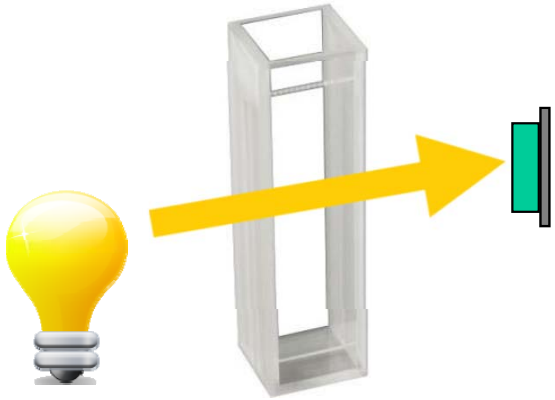
產品比較

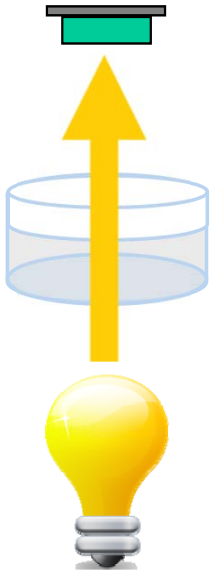
	Synergy H1	Synergy HTX	Synergy LX
主要特色			
全波長			
吸收光	•	•	•
螢光	top/bottom		
濾鏡式			
螢光	top	top/bottom	top
冷光	•	•	•
雷射光 Alpha excitation			
標準 Alpha excitation		•	
TRF & TR-FRET	•	(2° mode)	
偏極化螢光	•		
自動化/BioStack 相容性	•	•	
BioSpa™ 8 相容性	•		
雙試劑分注器相容性	•	•	

產品比較

	Synergy H1	Synergy HTX	Synergy LX
產品靈敏度			
Fluorescein - top	monos: 2.5 pM filters: 0.25 pM	5 pM	2 pM
Fluorescein - bottom	4 pM (monos)	5 pM	
ATP - luminescence	10 amol	30 amol/10 amol (lum-only model)	10 amol
Polarization - 1 nM fluorescein	1.2 mP std dev		
Europium	40 fM (filters)		
AlphaScreen		300 amol	
最快讀取速度 96-/384-well plates	11/22	14/26	12/23
產品規格			
Microplate types	1 to 384	1 to 384	1 to 384
氣體控制模組	•		
分注器模組	•	•	
Barcode reader option			
Take3™ Plate 相容性	•	•	•
溫度控制	to 45°C	to 50°C	
抗水氣凝集™	•	•	
螢光頻寬	mono: 16 nm filter: dependent	filter dependent	filter dependent

Gen5 光徑校正



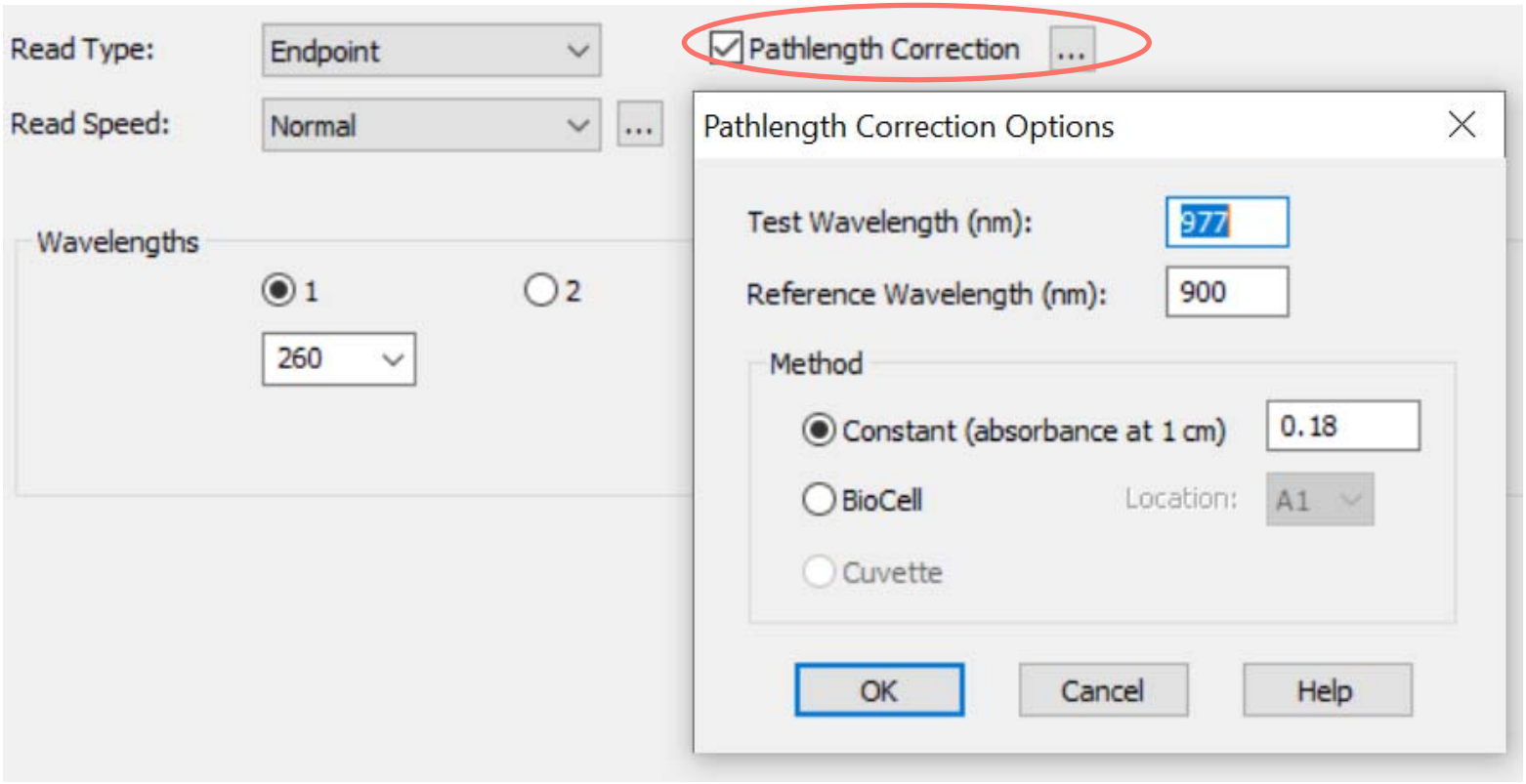


Cuvette spectrophotometer	Microplate reader
Horizontal	Vertical
1 cm	Variable

Gen5 光徑校正

- Water has a peak absorbance at 977 and 900 nm
- Pathlength = $(A977_{well} - A900_{well}) / (A977_{cuvette} - A900_{cuvette})$
  = K-factor
- The K-factor of water is close to 0.18
- Sample Pathlength (in cm) = $(A977_{well} - A900_{well}) / 0.18$
- Concentration = OD / Pathlength / Extinction coefficient
  = Corrected OD

Gen5 光徑校正


Read Type:
Endpoint
Pathlength Correction
...
Read Speed:
Normal
...
Wavelengths
1
2
260
Pathlength Correction Options
Test Wavelength (nm):
977
Reference Wavelength (nm):
900
Method
Constant (absorbance at 1 cm)
0.18
BioCell
Location:
A1
Cuvette
OK
Cancel
Help

Agilent

Trusted Answers

BioTek®

A part of Agilent